REMARKS/ARGUMENTS

Claims 69-78 are pending, claims 1-68 having been canceled and new claims 69-78 having been added. Support for new claim 69 is provided at, *e.g.*, p. 28, lines 13-32; p. 29, line 20 to p. 32, line 14; p. 10, lines 4-10; p. 13, line 27 to p. 14, line 2; and, p. 14, lines 9-18. Support for new claim 70 is provided at, *e.g.*, p. 14, lines 9-18. Support for new claim 71 is provided at, *e.g.*, pp. 13, lines 19-21; and, p. 14, lines 9-18. Support for new claim 72 is provided at, *e.g.*, p. 15, lines 1-3. Support for new claim 73 is provided at, *e.g.*, p. 15, lines 6-7. Support for new claim 74 is provided at, *e.g.*, p. 28, lines 20-21. Support for new claims 75 and 76 is provided at, *e.g.*, p. 28, lines 18-33. Support for new claim 77 is provided at, *e.g.*, p. 38, lines 32-33; p. 41, lines 1-9; and, p. 42, lines 8-12. Further support for new claims 69-78 is found throughout the specification as filed. Thus, the new claims contain no new matter.

New claims 69-78 of the present application are substantially copied from the claims of International Patent Application Publication No. WO 01/42306, published June 14, 2001 (U.S. Applicant: Chain, Benjamin; Applicant for all other states: Mindset Biopharmaceuticals (USA) Inc.) as shown in the following table.

Claim Pending in the Instant Application	**Substantially Copied Claims of WO 01/42306**
69	1
70	2
71	3
72	4
73	5
74	6

Claim Pending in the Instant Application	Substantially Copied Claims of WO 01/42306
75	7
76	8
77	10 and 11
78	12

An Information Disclosure Statement, the attached PTO/SB/08A form citing WO 01/42306 as cite no. 298, and a copy of WO 01/42306, are submitted herewith.

The instant application is a continuation of U.S. Application 09/723,544, filed November 28, 2000, now abandoned. We have amended the claims of the instant application to make them identical to those of U.S. Application 09/723,544 at the time of abandonment. We submit herewith a copy of the office action mailed August 11, 2003, for U.S. Application 09/723,544 for the convenience of the Examiner.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 650-326-2400.

Respectfully submitted,

Rosemarie L. Celli

Rosemarie L. Celli
Reg. No. 42,397

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60200878 v1

Form PTO 948 (Rev. 03/01) U.S. DEPARTMENT OF COMMERCE - Patent and Trademark Office Application No. 09/723544

NOTICE OF DRAFTSPERSON'S PATENT DRAWING REVIEW

The drawing(s) filed (insert date) 11-28-00 are:

A. ☐ approved by the Draftsperson under 37 CFR 1.84 or 1.152.

B. ☒ objected to by the Draftsperson under 37 CFR 1.84 or 1.152 for the reasons indicated below. The Examiner will require submission of new, corrected drawings when necessary. Corrected drawing must be sumitted according to the instructions on the back of this notice.

1. DRAWINGS. 37 CFR 1.84(a): Acceptable categories of drawings: Black ink. Color.
   - ____ Color drawings are not acceptable until petiton is granted. Fig(s) ____
   - ____ Pencil and non black ink not permitted. Fig(s) ____
2. PHOTOGRAPHS. 37 CFR 1.84(b)
   - ____ 1 full-tone set is required. Fig(s) ____
   - ____ Photographs may not be mounted. 37 CFR 1.84(e)
   - ____ Poor quality (half-tone). Fig(s) ____
3. TYPE OF PAPER. 37 CFR 1.84(e)
   - ____ Paper not flexible, strong, white, and durable. Fig(s) ____
   - ____ Erasures, alterations, overwritings, interlineations, folds, copy machine marks not accepted. Fig(s) ____
   - ____ Mylar, velum paper is not acceptable (too thin). Fig(s) ____
4. SIZE OF PAPER. 37 CFR 1.84(f): Acceptable sizes:
   - ____ 21.0 cm by 29.7 cm (DIN size A4)
   - ____ 21.6 cm by 27.9 cm (8 1/2 x 11 inches)
   - ____ All drawing sheets not the same size. Sheet(s) ____
   - ____ Drawings sheets not an acceptable size. Fig(s) ____
5. MARGINS. 37 CFR 1.84(g): Acceptable margins:

   Top 2.5 cm Left 2.5cm Right 1.5 cm Bottom 1.0 cm
   SIZE: A4 Size
   Top 2.5 cm Left 2.5 cm Right 1.5 cm Bottom 1.0 cm
   SIZE: 8 1/2 x 11
   Margins not acceptable. Fig(s) 1, 5, 6, 11, 13, 16, 17, 20
   - ____ Top (T) __X__ Left (L)
   - __X__ Right (R) ____ Bottom (B)
6. VIEWS. 37 CFR 1.84(h)
   REMINDER: Specification may require revision to correspond to drawing changes.
   Partial views. 37 CFR 1.84(h)(2)
8. ARRANGEMENT OF VIEWS. 37 CFR 1.84(i)
   - X Words do not appear on a horizontal, left-to-right fashion when page is either upright or turned so that the top becomes the right side, except for graphs. Fig(s) 16, 17, 18
9. SCALE. 37 CFR 1.84(k)
   - ____ Scale not large enough to show mechanism without crowding when drawing is reduced in size to two-thirds in reproduction. Fig(s) ____
10. CHARACTER OF LINES, NUMBERS, & LETTERS. 37 CFR 1.84(i)
    - ____ Lines, numbers & letters not uniformly thick and well defined, clean, durable, and black (poor line quality). Fig(s) ____
11. SHADING. 37 CFR 1.84(m)
    - ____ Solid black areas pale. Fig(s) ____
    - ____ Solid black shading not permitted. Fig(s) ____
    - ____ Shade lines, pale, rough and blurred. Fig(s) ____
12. NUMBERS, LETTERS, & REFERENCE CHARACTERS. 37 CFR 1.84(p)
    - ____ Numbers and reference characters not plain and legible. Fig(s) ____
    - ____ Figure legends are poor. Fig(s) ____
    - ____ Numbers and reference characters not oriented in the same direction as the view. 37 CFR 1.84(p)(1) Fig(s) ____
    - ____ English alphabet not used. 37 CFR 1.84(p)(2) Figs ____
    - ____ Numbers, letters and reference characters must be at least .32 cm (1/8 inch) in height. 37 CFR 1.84(p)(3) Fig(s) ____
13. LEAD LINES. 37 CFR 1.84(q)
    - ____ Lead lines cross each other. Fig(s) ____
    - ____ Lead lines missing. Fig(s) ____
14. NUMBERING OF SHEETS OF DRAWINGS. 37 CFR 1.84(t)

15270J-004762US
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/723,544	11/28/2000	Dale B. Schenk	15270J-004762US	9938

20350 7590 08/11/2003

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EXAMINER	
TURNER, SHARON L	
ART UNIT	PAPER NUMBER
1647	

DATE MAILED: 08/11/2003

Response Due 11/11/03, b3b
LAH/8-18-03

Please find below and/or attached an Office communication concerning this application or proceeding.

COPY

Copies sent on 8.28.03 to:

R. Celli
J. Liebeschuetz
6500US Binder

PTO-90C (Rev. 07-01)

Office Action Summary	Application No.	Applicant(s)
	09/723,544	SCHENK ET AL.
	Examiner	**Art Unit**
	Sharon L. Turner	1647

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *29 May 2003*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *69-95* is/are pending in the application.

4a) Of the above claim(s) *71,73,76 and 79-95* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *69,70,72,74,75,77 and 78* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☒ Claim(s) *69-95* are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *28 November 2000* is/are: a)☐ accepted or b)☒ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☒ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) 7.
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other:

DETAILED ACTION

Priority

1. If applicant desires priority under 35 U.S.C. 119(e) and 120 based upon a previously filed copending application, specific reference to the earlier filed application must be made in the instant application. This should appear as the first sentence of the specification following the title, preferably as a separate paragraph. The status of nonprovisional parent application(s) (whether patented or abandoned) should also be included. If a parent application has become a patent, the expression "now Patent No.____________" should follow the filing date of the parent application. If a parent application has become abandoned, the expression "now abandoned" should follow the filing date of the parent application.

Applicant's transmittal papers indicate priority as a CIP of 09/201,430 and of provisional 60/080,970. However, the first paragraph of the specification only reference the case as a continuation of 09/580,018 and as a CIP of 09/322,289.

2. Applicant has not complied with one or more conditions for receiving the benefit of an earlier filing date under 35 U.S.C. 119(e) and 120 as follows:

The later-filed application must be an application for a patent for an invention which is also disclosed in the prior application (the parent or original nonprovisional application or provisional application); the disclosure of the invention in the parent application and in the later-filed application must be sufficient to comply with the requirements of the first paragraph of 35 U.S.C. 112. See *Transco Products, Inc. v. Performance Contracting, Inc.*, 38 F.3d 551, 32 USPQ2d 1077 (Fed. Cir. 1994).

Instant specification is a continuation of 09/580,018 and shares the same disclosure. However, priority is claimed based upon 09/322,289 which disclosure apparently differs in that the '289 application fails to support instantly claimed chimeric peptides. Thus, the priority date awarded instant claims (effective filing date) is the filing date of the '018 case, 5-26-00. Traversal should include reference to the '289 application where support may be found for the instantly claimed chimeric peptides and immunogenic compositions.

Drawings

3. Figure 11 is objected to because the figure fails to specify the treatment groups via an appropriate key referencing those groups denoted by the symbols, i.e., open square, diamond, circle etc. A proposed drawing correction or corrected drawings are required in reply to the Office action to avoid abandonment of the application. The objection to the drawings will not be held in abeyance.

Election/Restriction

4. Applicant's election with traverse of Group I, to the extent of internal cleavage product Aβ1-3, T helper cell epitope derived from diphtheria toxoid, and N terminal of the first 3 amino acids of Aβ in Paper No. 11 is acknowledged. The traversal is on the ground(s) that particular claims are written in generic format and not Markush style format, that the subject matter is shared to the extent that portions of the beta amyloid sequence or T helper cell epitopes are similar in structure and that the multiple sequences should more properly be treated as species. This is not found persuasive because the claims are directed to mutiple patentably distinct inventions. The peptides

share uncommon structure in that the genus is variable. A search for any one particular chimeric molecule would not reveal all pertinent prior art to any other of the other chimeric molecules. The genus is not of a single shared structure but to multiple sub-generic elements. As the inventions so differ they can not be considered species that may be encompassed by a single search. Thus the inventions lack unity. Rejoinder would only be considered as to elements where the core structure is shared in common. The requirement is still deemed proper and is therefore made FINAL.

5. Claims 71, 73, 76 and 79-95 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to nonelected inventions and species, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in Paper No. 11.

Double Patenting

6. The non-statutory double patenting rejection, whether of the obviousness-type or non-obviousness-type, is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent. In re Thorington, 418 F.2d 528, 163 USPQ 644 (CCPA 1969); In re Vogel, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); In re Van Ornum, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); In re Longi, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); and In re Goodman, 29 USPQ2d 2010 (Fed. Cir. 1993).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(b) and (c) may be used to overcome an actual or provisional rejection based on a non-statutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.78(d).

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Claims 69-70, 72, 74-75 and 77-78 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-10 of copending Application No. 09/724,570, claims 89-98 of copending Application No. 09/723,927, claims 1-7, 44-45, 48 and 65 of copending Application No. 09/497,553, claim 65 of copending Application No. 09/724,477, claims 102-109 of copending Application No. 09/724,489, claims 33-37 of copending Application No. 09/580019, claims 1-10 of copending Application No. 09/585,817, 09/724,567, 09/724,575, 09/724,953, 09/724,570 and 09/979,952. Although the conflicting claims are not identical, they are not patentably distinct from each other because the instant claims are either anticipated by, or would have been obvious over the copending claims in each of the aforementioned applications.

In particular all claims are drawn to peptides, pharmaceutical or immunogenic compositions comprising peptides. Instant claims are viewed as being the broadest claims amongst the co-pending applications as the peptides comprise the broadest variability with respect to sequence structure and the immunogenic compositions merely comprise the peptide with either an acceptable carrier, excipient, diluent or adjuvant. The peptides of instant claims diverge from beta-amyloid or various plaque forming peptides as claimed in the copending cases. Thus, the copending peptides and/or peptide compositions are species or sub-genera of the instant peptides that are completely encompassed within the larger genus of instant claims.

Further, as all peptides are immunogenic to some extent and as all peptides for laboratory manipulation are provided to the artisan in some form of suitable carrier, excipient, diluent or adjuvant, the copending compositions and functional language as to providing an immune response would either anticipate or render obvious instant claims directed to peptides with T helper cell epitopes that effect immune responses and to

immunogenic compositions comprising the peptides. The particular compositions of the copending claims as to adjuvants are species that anticipate the compositions of instant claim 77. To the extent that the copending claims do not specifically recite alum as in claim 78, the artisan nevertheless recognizes that such is a suitable adjuvant choice for effecting an enhanced immune response with coadministration of a peptide. Thus, the copending claims are all directed to particular species or sub-genera of the instantly claimed larger genus claims. The copending claims if issued first would be completely encompassed within instant claims and the copending claims if issued first would serve as a species or subgenus capable of anticipating the broader claims of instant genus. Thus, the copending claims are subject to obviousness-type double patenting rejections.

This is a provisional obviousness type double patenting rejection because the conflicting claims have not in fact been patented.

Claim Rejections - 35 USC § 112

7. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

8. Claims 69-70, 72, 74-75 and 77-78 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This is a new matter rejection.

Instantly pending claims are as amended 6-14-02. In particular, the claims are newly drawn to chimeric peptides and immunogenic compositions. This amendment points to support for the recitations of new claim 69 at pp. 10, 13-14 and 28-32. However, review of the specification at such pages fails to provide support for the recitations as follows. Claim 69 as amended recites: A chimeric peptide having a first portion and a second portion, wherein the carboxyl terminus of the first portion is linked to the amino terminus of the second portion; and, wherein the first portion is from the free N-terminus of a naturally-occurring internal peptide cleavage product which, when naturally occurring in a mammal, is derived from a precursor protein or a mature protein and the second portion comprises a T helper cell epitope; or, wherein the first portion comprises a T helper cell epitope and the second portion is from the free C-terminus of said naturally occurring internal peptide cleavage product. The specification does not apparently support the breadth of peptides now contemplated.

In particular, no support is found for the breadth of peptides within "the first portion" as "from the free N-terminus of a naturally-occurring internal peptide cleavage product which, when naturally occurring in a mammal, is derived from a precursor protein or a mature protein", the particular linkage of the first portion and the second portion, and the breadth of peptides contemplated in the second portion as drawn to "T helper cell epitipes". Further with respect to the second portion of the claim, the specification does not apparently support the breadth of peptides wherein the second portion is from the "free C-terminus of said naturally occurring internal peptide cleavage product", the particular linkage and to the first portion and wherein the first portion has

"T helper cell epitopes". Further, the specification does not apparently support the recitation of "an immunologically effective amount" as recited in claims 77-78 or for the recitation of "alum" in claim 78. Thus, the recitations constitute new matter absent particular evidence for their support.

9. Claims 69-70, 72, 74-75 and 77-78 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This is a written description rejection.

The specification describes a polypeptide sequence consisting of beta-amyloid 1-42 and beta amyloid 1-5 conjugated to sheep anti-mouse IgG which are disclosed as exhibiting beta amyloid plaque clearing activity in PDAPP mice, see in particular pp. 60-64 of the specification. It is also apparent from the specification that the artisan was in possession of other particular beta amyloid peptides and peptide conjugates capable of stimulating antibody reactive to beta-amyloid, see in particular specification p. 60-61. While not capable of clearing amyloid plaques, these peptides and/or immunogenic compositions were useful for the purpose of stimulating beta-amyloid specific antibodies for detection of Abeta peptides and/or Alzheimer's plaques.

The claims are also directed to peptides comprising a second portion that is a suitable "T helper cell epitope". T helper cell epitopes are generally known to be peptides capable of stimulating the release of lymphokines that activate B cells thus stimulating an enhanced B cell (antibody) response. While the artisan is apprised of

particular T helper cell epitopes as disclosed in the prior art, the artisan recognizes that a substantial number of such peptides capable of eliciting Th function remain to be determined or discovered. Moreover, the specification and art fails to disclose any definitive structure or definitive assay whereby Th cell activity is determined. Instead the structures amongst different peptides, with different MHC molecules and different art recognized assays (cytokine stimulation, T cell proliferation etc.) for determining function vary substantially. In addition, the specification does not apparently provide for any particular diphtheria T helper cell epitopes, describe their particular characteristics either structurally or functionally. While the artisan is provided with particularly known T helper cell epitopes of the prior art including as disclosed at p. 28, the claims do not adequately describe by structure all known T-helper cell epitopes amongst all possibly known sequences. The specification and claims fail to delineate those structural and/or functional requirements of peptides described as T helper cell epitopes. While the artisan has multiple assays to measure T cell reactivity or antibody production (suitable measurements of T helper cell eptiope activity), see for example specification p. 10, lines 12-29, no particular structural and or functional features or assays are denoted as being definitive of the recitation as claimed.

The claims encompass peptides comprising a "first portion from the free N-terminus of a naturally-occurring internal peptide cleavage product which, when naturally occurring in a mammal, is derived from a precursor protein or a mature protein" and a second portion comprising "a T helper cell epitope ". Yet, the specification fails to adequately describe that which is "the free N-terminus of a naturally

–occurring internal peptide cleavage product which, when naturally occurring in a mammal is derived from a precursor protein or a mature proteins," because the specification and claims fail to distinguish the required structural features or any method of determining the suitable peptides or peptide sequences. The artisan must be able to determine if any particular sequence meets this limitation of the claims, including whether a peptide is suitably derived from a naturally-occurring molecule, encompassing allelic forms. Yet to the extent that the sequence is merely derived from a multitude of mammalian peptides, naturally occurring variants and to sequences that are unlimited in length, the artisan is left with little understanding of either the structural and/or functional features required. With respect to the free N-terminus, the specification is only exemplary to a few beta-amyloid sequences.

Thus the specification provides inadequate written description with respect to these recitations. The instant disclosure of a few single polypeptides with the instantly disclosed specific activities, does not adequately support the scope of the claimed genus, which encompasses a substantial variety of subgenera. It is further noted that no function is required of the chimeric peptides. As to the immunogenic compositions, no particular immune function is denoted as being required. A genus claim may be supported by a representative number of species as set forth in *Regents of the University of California v Eli Lilly & Co,* 119F3d 1559, 1569, 43 USPQ2d 1398, 1406 (Fed. Cir. 1997), which states:

"To fulfill the written description requirement, a patent specification must describe an invention and do so in sufficient detail that one skilled in the art can clearly conclude that "the inventor invented the claimed invention". Lockwood v. American Airlines, Inc.,

107 F.3d 1565, 1572, 41 USPQ2d 1961, 1966 (1997); In re Gosteli, 872 F.2d 1008, 1012, 10 USPQ2d 1614, 1618 (Fed. Cir. 1980) ("[T]he description must clearly allow persons of ordinary skill in the art to recognize that [the inventor] invented what is claimed.") Thus, an applicant complies with the written description requirement "by describing the invention, with all its claimed limitations, not that which makes it obvious," and by using "such descriptive means as words, structures, figures, diagrams, formulas, etc., that set forth the claimed invention." Lockwood, 107 F.3d 1565, 1572, 41 USPQ2d at 1966.

An adequate written description of a DNA, such as the cDNA of the recombinant plasmids and microorganisms of the '525 patent, "requires a precise definition, such as by structure, formula, chemical name, or physical properties," not a mere wish or plan for obtaining the claimed chemical invention. Fiers v. Revel, 984 F.2d 1164, 1171, 25 USPQ2d 1601, 1606 (Fed. Cir. 1993). Accordingly, "an adequate written description of a DNA requires more than a mere statement that it is part of the invention and reference to a potential method for isolating it; what is required is a description of the DNA itself." Id at 1170, 25 USPQ2d at 1606."

A description of a genus may be achieved by means of a recitation of a representative number of members falling within the scope of the genus, or of a recitation of structural features common to the genus, which features constitute a substantial portion of the genus. In the instant case the claims appear to encompass peptides in which the first portion is of nearly unlimited structure without function while the second portion is loosely limited to several (as generically claimed) functional recitations without any particular structural guidance. Thus, neither the specification, prior art nor claims provides sufficient structural and/or functional correlative teachings to enable one of skill to identify the polypeptides encompassed by either the first portion

or the second portion. Without suitable description of the first and second portion members, the chimeric peptides and immunogenic compositions lack adequate written description support such that the artisan is apprised that applicant was in possession of the invention claimed with peptides comprising such first and second portions. There is no fulfilling structural and/or functional requirements for the chimeric peptides and their immunogenic compositions.

10. Claims 69-70, 72, 74-75 and 77-78 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for reducing beta-amyloid plaque burden in PDAPP transgenic mice which over-express amyloid by administration of beta amyloid 1-42 peptide (AN1792) and beta amyloid 1-5 peptide conjugated to sheep anti-mouse IgG, see in particular pp. 60-64, or for providing particular peptides capable of stimulating beta-amyloid specific antibodies, does not reasonably provide enablement for the chimeric peptides or immunogenic compositions as claimed. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected to make and use the invention commensurate in scope with these claims.

The specifications disclosure is insufficient to enable one skilled in the art to practice the invention as broadly claimed without undue experimentation. The factors relevant to this discussion include the quantity of experimentation necessary, the lack of working examples, the unpredictability of the art, the lack of sufficient guidance in the specification and the breadth of the claims.

The claims are drawn to various chimeric peptides and immunogenic compositions. The peptides and compositions are disclosed as useful in the prevention

or treatment of diseases, particularly of the nervous system including diseases associated with amyloid deposits of Aβ or for stimulating amyloid specific antibodies. The utility of the peptides and compositions is based upon findings which show particular strategies of targeting plaque removal via antigen or antibody administration and for labeling amyloid plaques. Evidence that such therapy can be effective in the removal of amyloid plaque burden or in detection of amyloid plaques is exhibited in applicant's specification.

However, what the specification does not teach is the scope of antigen/antibody variability effective to promote plaque removal or clearance or that enables amyloid specific antibody production. In particular neither instant specification nor the art recognizes treatment or detection with the broad scope of chimeric peptides and immunogenic compositions now claimed wherein the multitude of the compositions differ substantially in sequence structure, length and the ability to mediate particular immune responses.

The specification teaches at pp. 60-64 that administration of aggregated beta amyloid 1-42 peptide (AN1792) and beta amyloid 1-5 peptide conjugated to sheep anti-mouse IgG is effective at reducing beta-amyloid levels within the brains of mice which are transgenic for PDAPP. In particular these results are noted at p. 63, line 30-p 64, line 20. However, the specification further reveals that conjugates of other beta-amyloid peptides were insufficient to produce any reduction in beta-amyloid plaque accumulation. Such results evidence the unpredictability in the art with respect to effecting an adequate immune response with members of the chimeric peptides and

immunogenic compositions claimed. The specification fails to evidence any means for determining a priori which of the encompassed sequences reliably and predictably provide for such beneficial use.

Moreover, the claims are directed to peptides comprising a "first portion from the free N-terminus of a naturally-occurring internal peptide cleavage product which, when naturally occurring in a mammal, is derived from a precursor protein or a mature protein" and a second portion comprising "a T helper cell epitope ". Yet, the specification fails to adequately describe that which is "the free N-terminus of a naturally –occurring internal peptide cleavage product which, when naturally occurring in a mammal is derived from a precursor protein or a mature proteins," because the specification and claims fail to distinguish the required structural features. The artisan must be able to determine if any particular sequence meets this limitation of the claims. Yet to the extent that the sequence is merely derived from a multitude of mammalian peptides and is unlimited in length, the artisan is left with little understanding of either the structural or functional features required. With respect to the first portion, the specification is only exemplary to a few beta-amyloid sequences.

Moreover, while the artisan is provided with particularly known T helper cell epitopes of the prior art, the claims do not adequately describe by structure all known T-helper cell epitopes amongst all possibly known sequences. The specification and claims fail to delineate those structural and/or functional requirements of the peptides described as T helper cell epitopes. While the artisan has multiple assays to measure T cell reactivity, see for example specification p. 10, lines 12-29, no particular structural

and or functional features or assays are denoted or recognized in the art as being definitive of the recitation. Such is akin to a single means claim i.e., where a means recitation does not appear in combination with another recited element of means and is subject to an undue breadth rejection under 35 USC 112, first paragraph because the specification at most would only disclose those means known to the inventor at the time of the invention, see in particular MPEP 2164.08(a).

The skilled artisan further recognizes that protein chemistry is an unpredictable area of biotechnology. Proteins with deletion, insertion or substitution/replacement of single amino acid residues may lead to both structural and functional changes in biological activity and immunological recognition, see in particular Skolnick et al., Trends in Biotech.,18(1):34-39, 2000. For example, Jobling et al, Mol. Microbiol., 1991, 5(7):1755-67 teaches a panel of single amino acid substitutions by oligonucleotide directed mutagenesis which produce proteins that differ in native conformation, immunological recognition, binding and toxicity. The skilled artisan further recognizes that immunological responses depend upon the particular antibody molecule. In particular, antibody recognition occurs via antibody variable and constant regions, see in particular Benjamini, Wiley Liss, 1991, pp. 49-65 and Table 5.1. Each antibody molecule is unique with respect to it antigen and the biological function which it is capable of eliciting within a host, see in particular Benjamini, pp. 49-50 and Table 5.1. Thus, both biological function and immunological recognition are unpredictable properties which must be experimentally determined.

Thus, the specification does not enable the broad scope of the claims that encompasses various chimeric peptides and immunogenic compositions because the specification does not teach the specificity required. Moreover, while the artisan recognizes that particular peptide peptide immunogens may be "universally" useful for stimulating an immune response i.e., as adjuvant or Th eptiopes may be, these peptides are not immediately provided but instead are generically claimed by the recitation of a T helper cell epitope.

Instead the artisan must make and then determine the utility of any particular chimeric peptide or immunogenic composition. While it would not be undue to make any particular peptide sequence possible, such does not fulfill the ability to use any peptide sequence possible. The specification does not correlate the breadth of the peptides encompassed to their respective uses. For example, given the breadth of the sequences it would not be surprising that particular peptides encompassed by the claims would be incapable of stimulating any reactivity to beta-amyloid. As the specification is written in context of this utility for diagnosis, detection and development of pharmaceuticals for the treatment of Alzheimer's, the artisan would be left unapprised of such divergent molecules' respective use.

Thus, for the aforementioned reasons, applicants have not provided sufficient guidance to enable one skilled in the art to make and use the claimed chimeric peptides and immunogenic compositions in a manner reasonably correlated with the scope of the claims. The scope of the claims must bear a reasonable correlation with the scope of enablement (In re Fisher, 166 USPQ 19 24 (CCPA 1970)). Without such guidance, the

changes which can be made and still maintain activity/utility is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See Ex parte Forman, 230 USPQ 546 (Bd. Pat. App. & Int. 1986).

In view of the quantity of experimentation necessary, the lack of working examples, the unpredictability of the art, the lack of sufficient guidance in the specification and the breadth of the claims, it would take undue experimentation to make and use the claimed invention.

Claim Rejections - 35 USC § 102 and 103

11. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless --
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

12. Claim 69, 75 and 77 are rejected under 35 U.S.C. 102(b) as being anticipated by Sad et al., Vaccine, 11(11):1145-1149, 1993.

Sad et al., teach chimeric peptide conjugates of syntheic gonadotrophin-releasing hormone and diphtheria T helper cell epitopes. In particular the Sad peptide meets instant limitations in that the peptide is chimeric, possesses a first portion GnRH comprising a free N-terminus of the naturally occurring internal peptide cleavage product derived form the mature GnRH protein (after signal sequence cleavage) and a

second portion comprising diphtheria toxin T helper cell epitopes, see in particular p. 1146, columns 1-2. The reference also notes GnRH conjugates to diphtheria toxin, see in particular p. 1145, column 2, first paragraph. The peptide conjugates were administered as saline emulsified freund's adjuvant compositions or as adsorbed peptides on calcium phosphate, see in particular p. 1146-1147. Thus, the reference teachings anticipate the claimed invention.

13. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(f) or (g) prior art under 35 U.S.C. 103(a).

14. Claims 69-70, 72, 74, 75 and 77-78 are rejected under 35 U.S.C. 103(a) as being unpatentable over Suzuki et al., US 5,750,349 (May 12, 1998), Stevens et al., US 4,713,366(Dec. 15, 1997) and Lehrer et al., US 5,464,823 (Nov. 7, 1995) as evidenced by Thomas et al., US 6,284,533 (Sept. 4, 2001).

Suzuki et al., teach antibodies to beta-amyloid generated via the use of peptide immunogens comprising various portions or derivatives of beta-amyloid, see in particular Disclosure of Invention, columns 3-13. In particular the portions include the N- or C-terminus of beta-amyloid (a naturally occurring internal peptide cleavage product which when naturally occurring in a mammal is derived from a precursor protein or a mature protein) derived from the mammalian protein amyloid precursor protein, see in particular Abstract and Background Art, columns 1-3 in the context of claims. Such immunogen peptides are noted in SEQ ID NO's:1-7 and 10 as having a portion of beta amyloid residues 1-3, Asp-Ala-Glu as recited in claim 72. The peptides antigens are produced bound to or adsorbed to appropriate carriers for immunization including with adjuvants and are immunogenically effective to produce antibodies, see in particular column 15, lines 1-53 (claim 77). Suzuki teaches that the antibodies derived from the disclosed immunogens are useful in the detection and diagnosis of diseases related to beta-amyloids including Alzheimer's and for the development of preventive-therapeutic compositions.

However, Suzuki et al., do not teach the chimeric peptide claimed wherein the carboxyl terminus of the first portion is linked to a T helper cell epitope (claim 69), binds multiple MHC molecules (claim 74), is derived from diphtheria toxoid (claim 75) and wherein the adjuvant is alum (claim 78)

Stevens et al., 4,713,366 teach antigenic modification of polypeptides wherein the sequence is coupled or linked to diphtheria toxoid and immunogenic compositions wherein the adjuvant is alum, see in particular column 23, lines 12-53, column 37, lines 3-50, column 38, line 51-column 39, line 7, column 44, line 42-column 45, line 4, column 46, lines 35-57, column 79-80, Table 13, column 81, lines 39-57, column 82, lines 66-column 83, line 3 and column 87, lines 36-55.

Lehrer et al., US 5,464,823 similarly teach methods for producing antibodies including via linkage with suitable carriers including diphtheria toxoid. In particular, at column 9, lines 39-58 states, "Antibodies to the protegrins of the invention may also be produced using standard immunological techniques for production of polyclonal antisera and, if desired, immortalizing the antibody-producing cells of the immunized host for sources of monoclonal antibody production. Techniques for producing antibodies to any substance of interest are well known. It may be necessary to enhance the immunogenicity of the substance, particularly as here, where the material is only a short peptide, by coupling the hapten to a carrier. Suitable carriers for this purpose include substances which do not themselves produce an immune response in the animal to be administered the hapten-carrier conjugate. Common carriers used include keyhole limpet hemocyanin (KLH), diphtheria toxoid, serum albumin, and the viral coat protein of rotavirus, VP6. Coupling of the hapten to the carrier is effected by standard techniques such as contacting the carrier with the peptide in the presence of a dehydrating agent such as dicyclohexylcarbodiimide or through the use of linkers such as those available through Pierce Chemical Company, Chicago, Ill."

While Stevens et al., and Lehrer et al., are silent as to diphtheria toxin's

properties as T helper cell epitiopes and the ability to bind multiple MHC molecules, such is evidenced by Thomas et al., US 6284533.

In particular, Thomas teaches particular peptides such as diphtheria toxoid that provide universal T helper cell epitopes capable of binding multiple MHC molecules as follows:

at column 5, lines 21-58;

"The immunogenic fusion polypeptide encoded on a plasmid as described herein comprises a T cell epitope portion and a B cell epitope portion. A T cell epitope portion encoded on the plasmid of this invention comprises a non-endogenous CETP protein, or fragment thereof, that contains a broad range or "universal" helper T cell epitope which binds the antigen presenting site of multiple (i.e., 2, 3, 4, 5, 6 or more) class II major histocompatibility (MHC) molecules and can form a tertiary complex with a T cell antigen receptor, i.e., MHC:antigen:T cell antigen receptor. By "non-endogenous CETP protein" is meant a protein which is not the endogenous CETP of the individual who is to be administered a plasmid of this invention. Such non-endogenous CETP proteins, or fragments thereof, useful as T cell epitope portions of the immunogenic fusion polypeptide encoded by plasmids of this invention include tetanus toxoid (particularly peptides of tetanus toxoid having amino acid sequences of amino acids 2-15 of SEQ ID NO:7 and amino acid sequence of SEQ ID NO:10); diphtheria toxin (particularly peptides having amino acid sequences of amino acids 271-290, 321-340, 331-350, 351-370, 411-430, and 431-450 of SEQ ID NO:9); class II MHC-associated invariant chain; influenza hemagglutinin T cell epitope; keyhole limpet hemocyanin (KLH); a protein from known vaccines including pertussis vaccine, the Bacile Calmette-Guerin (BCG) tuberculosis vaccine, polio vaccine, measles vaccine, mumps vaccine, rubella vaccine, and purified protein derivative (PPD) of tuberculin; and also synthetic peptides which bind the antigen presenting site of multiple class II histocompatibility molecules, such as those containing natural amino acids described by Alexander et al. (Immunity, 1: 751-761 (1994)). When attached to a CETP B cell epitope portion, the T cell epitope portion enables the immunogenic fusion polypeptide to break tolerance in order for antibodies to be made that react with endogenous CETP. By "breaking tolerance" is meant forcing an organism to mount an immune response to a protein, such as endogenous CETP, that the organism does not normally find immunogenic.

at column 10, lines 26-40;

Broad range antigenic helper T cell epitopes are known in the art. These include, for example, epitopes of tetanus toxoid (TT) and diphtheria toxoid (DT) (see, for example, Panina-Bordignon, P., et al., Eur. J. Immunol., 19: 2237-2242 (1989) (characterization of universal tetanus toxoid helper T cell epitope peptides); Etlinger, H.,

Immunol. Today, 13: 52-55 (1992); Valmori, D., et al., J. Immunol., 149: 717-721 (1992) (use of universal TT epitopes in candidate anti-malarial vaccine); Raju et al., Eur. J Immunol., 25: 3207-3214 (1995) (broad range T cell epitopes of DT); Talwar, G. P., et al., Proc. Natl. Acad. Sci. USA, 91: 8532-8536 (1994) (use of TT and DT as universal epitopes in anti-human chorionic gonadotropin vaccine); Talwar, G. P., et al., Proc. Natl. Acad Sci. USA, 91: 8532-8536 (1994)).

at column 10, line 57-column 11, line 14;

Plasmids of this invention may encode a variety of non-endogenous CETP proteins, or fragments thereof, such as tetanus toxoid, particularly peptides of tetanus toxoid having amino acid sequences of amino acids 2-15 of SEQ ID NO:7 (a corresponding nucleotide coding sequence is nucleotides 13-54 of SEQ ID NO:5) and amino acid sequence of SEQ ID NO:10. Another source of universal or broad range T cell epitopes useful in the plasmids of this invention is diphtheria toxin, particularly peptides having amino acid sequences of amino acids 271-290, 321-340, 331-350, 351-370, 411-430, and 431-450 of SEQ ID NO:9. An example of corresponding nucleotide sequences encoding these broad range T cell epitopes from diphtheria toxin are nucleotides 811-870, 961-1020, 991-1050, 1051-1110, 1231-1290, and 1291-1350 of SEQ ID NO:8, respectively. Other sources of universal or broad range T cell epitopes that may be encoded on plasmids of this invention include, but are not limited to, class II MHC-associated invariant chain; hemagglutinin; keyhole limpet hemocyanin (KLH); a protein from known vaccines including pertussis vaccine, the Bacile Calmette-Guerin (BCG) tuberculosis vaccine, polio vaccine, measles vaccine, mumps vaccine, rubella vaccine, and purified protein derivative (PPD) of tuberculin; and also synthetic peptides as described by Alexander et al. (1994).

at column 20-21, Example IV;

The results of the above experiment using a rabbit model for atherosclerosis indicate that the plasmid-based vaccines of this invention may be used to prevent or treat atherosclerosis in other vertebrates. By analogy to the treatment for inhibiting atherosclerosis in rabbits illustrated in Example III, similar plasmid constructs may be made for other vertebrates, including humans. Such plasmids encode an immunogenic fusion polypeptide comprising a universal or broad range T cell epitope, such as from tetanus toxoid or diphtheria toxoid, linked in the same reading frame to at least one, more preferably two, B cell epitopes of the endogenous CETP of the individual. An example of a plasmid-based vaccine for endogenous human CETP contains a DNA sequence encoding a translation initiating methionine linked to a TT polypeptide, such as in nucleotides 10-54 of SEQ ID NO:5, which is linked in the same reading frame (with or without intervening linker sequences) to a DNA sequence encoding regions of human CETP analogous to those used in the rabbit CETP plasmid-based vaccine, such as nucleotides 1045-1101 and 1381-1428 of SEQ ID NO:3 encoding amino acids 349-367 and 461-476 of SEQ ID NO:4, respectively. Preferably, the DNA sequence in the plasmid for use as a vaccine against human endogenous CETP also includes regions as shown in FIG. 5, such as translational start and stop codons and flanking

restriction endonuclease sites that are commonly employed for plasmid construction and gene expression.

Thus, one of skill in the art would be motivated to modify the peptide immunogens of Suzuki et al., to provide chimeric peptides wherein the beta-amyloid portion of Suzuki is linked to the diphtheria toxoid portion of Stevens or Lehrer. Such modification results in a chimeric peptide comprising a first portion wherein the first portion is linked to the amino terminus of the free N-terminus of a naturally occurring internal peptide cleavage product which when naturally occurring in a mammal is derived from a precursor protein and a second portion comprising a T helper cell epitope capable of binding multiple MHC molecules. In addition, Stevens teaches that such immunogenic peptides may be provided in an immunogenically effective amount to produce antibodies via administration with adjutants including alum. One of skill in the art would have been motivated to make such modifications in order to enhance the immunogenicity of the beta-amyloid peptides in order to obtain antibodies suitable for detection and diagnosis of Alzheimer's disease as taught via Suzuki, Lehrer, and Stevens cumulatively. Thomas evidences that diphtheria toxoid would be a suitable carrier for provoking antibody responses via providing T helper cell epitopes that bind to multiple MHC molecules. One of skill in the art would have expected success using such combinations based upon the reference's teachings of enhanced immunogenicity and the optimal carrier properties of diphtheria toxin with alum as taught by Stevens and Lehrer. Thus, the cumulative reference teachings anticipate the claimed invention.

Status of Claims

15. No claims are allowed.

16. Any inquiry of a general nature or relating to the status of this general application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Papers relating to this application may be submitted to Technology Center 1600, Group 1640 by facsimile transmission. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989). Should applicant wish to FAX a response, the current FAX number for Group 1600 is (703) 308-4242.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Sharon L. Turner, Ph.D. whose telephone number is (703) 308-0056. The examiner can normally be reached on Monday-Thursday from 8:00 AM to 6:30 PM. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Kunz, can be reached at (703) 308-4623.

Sharon L. Turner, Ph.D.
August 8, 2003

Lorraine Spector

LORRAINE SPECTOR
PRIMARY EXAMINER

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Notice of References Cited	Application/Control No. 09/723,544	Applicant(s)/Patent Under Reexamination SCHENK ET AL.	
	Examiner Sharon L. Turner	Art Unit 1647	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-6,284,533	09-2001	Thomas, Lawrence J.	435/320.1
	B	US-4,713,366	12-1987	Stevens, Vernon C.	514/13
	C	US-5,464,823	11-1995	Lehrer et al.	514/13
	D	US-5,750,349	05-1998	Suzuki et al.	435/7.1
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

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	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Skolnick et al., Trends in Biotech.,18(1):34-39, 2000
	V	Jobling et al, Mol. Microbiol., 1991, 5(7):1755-67
	W	Benjamini, Wiley Liss, 1991, pp. 49-65 and Table 5.1
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

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Sheet 1 of 1

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Examiner	Cite No.[1]	Document Number — Number Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
SS	242	US 60/168,594		Chain	

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Examiner Initials*	Cite No.[1]	Foreign Patent Document — Country Code[3]	Number[4]	Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T[6]
SS	243	WO	01/39796	A2	06-07-2001			

Examiner Signature	S Turner	Date Considered	7-28-03

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[1] Unique citation designation number. [2] See attached Kinds of U.S. Patent Documents. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST. 16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

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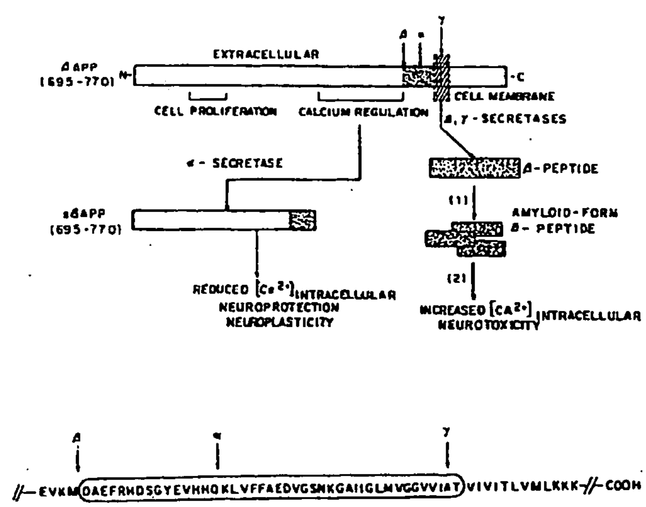
(54) Title: CHIMERIC PEPTIDES AS IMMUNOGENS, ANTIBODIES THERETO, AND METHODS FOR IMMUNIZATION USING CHIMERIC PEPTIDES OR ANTIBODIES



(57) Abstract: The invention provides a chimeric peptide or mixture of chimeric peptides that can be formulated as an immunizing composition and used in a method for immunization of a mammal against an internal peptide cleavage product derived from a precursor or mature protein, for which the peptide cleavage product and the precursor or mature protein are self molecules. The chimeric peptide or peptides have an end-specific B cell epitope from a naturally-occurring internal peptide cleavage product of a precursor or mature protein, as a free N- or C- terminus, fused with or without spacer residues to a T helper cell epitope derived from a living source different from that of the internal peptide cleavage product.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

CHIMERIC PEPTIDES AS IMMUNOGENS, ANTIBODIES THERETO, AND METHODS FOR IMMUNIZATION USING CHIMERIC PEPTIDES OR ANTIBODIES

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims the benefit of priority from U.S. provisional application no. 60/169,687, filed December 8, 1999, the entire contents of which are hereby incorporated by reference.

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to a chimeric peptide immunogen containing a B cell epitope joined to a T cell epitope from a different source, immunizing compositions containing the chimeric peptide, and a method for immunization using same.

Description of the Background Art

A major histopathological hallmark of Alzheimer's Disease (AD) is the presence of amyloid deposits within neuritic and diffuse plaques in the parenchyma of the amygdala, hippocampus and neocortex (Glenner and Wong, 1984; Masters et al., 1985; Sisodia and Price, 1995). Amyloid is a generic term that describes fibrillar aggregates that have a common β-pleated structure. These aggregates exhibit birefringent properties in the presence of Congo red and polarized light (Glenner and Wong, 1984). The diffuse plaque is thought to be relatively benign in contrast to the neuritic plaque which appears to be strongly correlated with reactive and degenerative processes (Dickson et al., 1988; Tagliavini et al., 1988; Yamaguchi et al., 1989; Yamaguchi et al., 1992). The principal component of neuritic plaques is a 42 amino acid residue amyloid-β (Aβ) peptide (Miller et al., 1993; Roher et al., 1993) that is derived from the much larger β-amyloid precursor protein, βAPP (or APP) (Kang et al., 1987). Two major C-terminal variants of amyloid-β peptide, Aβ 1-40 ending at Val40 and Aβ 1-42(43) ending at Ala42 or Thr43, proteolytically cleaved from βAPP, were found in amyloid deposits (Miller et al., 1993; Roher et al., 1993). Aβ

1-42 is produced less abundantly than the 1-40 Aβ peptide (Haass et al., 1992; Seubert et al., 1992), but the preferential deposition of Aβ1-42 results from the fact that this COOH-extended form is more insoluble than 1-40 Aβ and is more prone to aggregate and form anti-parallel β-pleated sheets (Joachim et al., 1989; Halverson et al., 1990; Barrow et al., 1992; Burdick et al., 1992; Fabian et al., 1994). Aβ1-42 can seed the aggregation of Aβ 1-40 (Jarrett and Lansbury 1993). Iwatsubo et al., (1996) and Saido et al., (1996) further reported that other variant amyloid-β peptides, Aβ 3(pyroglutamate)-42, Aβ 11(pyroglutamate)-42, Aβ 17-42, Aβ 1(D-Asp)-42, and Aβ 1(L-isoAsp)-42 were also found to be present in amyloid deposits in the brain.

The APP gene was sequenced and found to be encoded on chromosome 21 (Kang et al., 1987). Expression of the APP gene generates several Aβ-containing isoforms of 695, 751 and 770 amino acids (Figure 1), with the latter two βAPP containing a domain that shares structural and functional homologies with Kunitz serine protease inhibitors (Kang et al., 1987; Kitaguchi et al., 1988; Ponte et al., 1988; Tanzi et al., 1988; Konig et al., 1992). The functions of βAPP in the nervous system remain to be defined, although there is increasing evidence that βAPP has a role in mediating adhesion and growth of neurons (Schubert et al., 1989; Saitoh et al., 1994; Saitoh and Roch, 1995) and possibly in a G protein-linked signal transduction pathway (Nishimoto et al., 1993). In cultured cells, βAPPs mature through the constitutive secretory pathway (Weidemann et al., 1989; Haass et al., 1992; Sisodia 1992) and some cell-surface-bound βAPPs are cleaved within the Aβ domain by an enzyme, designated α-secretase, (Esch et al., 1990), an event that precludes Aβ amyloidogenesis (Figure 1). Several studies have delineated two additional pathways of βAPP processing that are both amyloidogenic: first an endosomal/lysosomal pathway generates a complex set of βAPP-related membrane-bound fragments, some of which contain the entire Aβ sequence (Haass et al., 1992; Golde et al., 1992); and second, by mechanisms that are not fully understood, Aβ 1-40 is secreted into the

conditioned medium and is present in cerebrospinal fluid *in vivo* (Haass et al., 1992; Seubert et al., 1992; Shoji et al., 1992; Busciglio et al., 1993). Lysosomal degradation is no longer thought to contribute significantly to the production of Aβ (Sisodia and Price, 1995). The proteolytic enzymes responsible for the cleavages at the NH_2 and COOH termini of Aβ termed β and γ (Figure 1), respectively, have not been identified. Until recently, it was generally believed that Aβ is generated by aberrant metabolism of the precursor. The presence, however, of Aβ in conditioned medium of a wide variety of cells in culture and in human cerebrospinal fluid indicate that Aβ is produced as a normal function of cells.

If amyloid deposition is a rate-limiting factor to produce AD, then all factors linked to the disease will either promote amyloid deposition or enhance the pathology that is provoked by amyloid. The likelihood of amyloid deposition is enhanced by trisonomy 21 (Down's syndrome) (Neve et al., 1988; Rumble et al., 1989), where there is an extra copy of the APP gene, by increased expression of APP, and by familial Alzheimer's Disease (FAD)-linked mutations (Van Broeckhoven et al., 1987; Chartier-Harlin et al., 1991; Goate et al., 1989; Goate et al., 1991; Murrell et al., 1991; Pericak-Vance et al., 1991; Schellenberg et al., 1992; Tanzi et al., 1992; Hendricks et al., 1992; Mullan et al., 1992). Some of these mutations are correlated with an increased total production of Aβ (Cai et al., 1993; Citron et al., 1992) or specific overproduction of the more fibrillogenic peptides (Wisniewski et al., 1991; Clements et al., 1993; Suzuki et al., 1994) or increased expression of factors that induce fibril formation (Ma et al., 1994; Wisniewski et al., 1994). Collectively, these findings strongly favor the hypothesis that amyloid deposition is a critical element in the development of AD (Hardy 1992), but of course they do not preclude the possibility that other age-related changes associated with the disease, such as paired helical filaments, may develop in parallel rather than as a result of amyloid deposition and contribute to dementia independently.

The main focus of researchers and the principal aim of those associated with drug development for AD is to reduce the amount of Aβ deposits in the central nervous system (CNS). These activities fall into two general areas: factors affecting the production of Aβ, and factors affecting the formation of insoluble Aβ fibrils. A third therapeutic goal is to reduce inflammatory responses evoked by Aβ neurotoxicity.

With regards to the first, a major effort is underway to obtain a detailed understanding of how newly synthesized βAPP is processed for insertion into the plasma membrane and to identify the putative amyloidogenic secretases that have been assigned on the basis of sites for cleavage in the mature protein. From a pharmacological perspective, the most direct way of reducing the production of Aβ is through direct inhibition of either β or γ secretase. No specific inhibitors are currently available although a number of compounds have been shown to indirectly inhibit the activities. Bafilomycin, for example, inhibits Aβ production with an EC_{50} of about 50 nM (Knops et al., 1995; Haass et al., 1995), most likely through its action as an inhibitor of vascular H^+ATPase co-localized in vesicles with the Aβ secretase. Another compound, MDL28170, used at high concentrations appears to block the activity of γ secretase Higaki et al., 1995). It is generally hoped that the identification of the β or γ secretases might lead to the synthesis of specific protease inhibitors to block the formation of amyloidogenic peptides. It is not known, however, whether these enzymes are specific for βAPP or whether they perhaps have other important secretory functions.

Similarly, problems of target and targeting specificity will be encountered through any attempt to interfere with signal transduction pathways that may determine whether processing of βAPP is directed through the amyloidogenic or non-amyloidogenic pathways. Moreover, these signal transduction mechanisms still need to be identified. In conclusion, present understanding of the complex and varied underlying molecular mechanisms leading to overproduction of Aβ offers little hope for selective targeting by pharmacological agents.

Given that neurotoxicity appears to be associated with β-pleated aggregates of Aβ, one therapeutic approach is to inhibit or retard Aβ aggregation. The advantage of this approach is that the intracellular mechanisms triggering the overproduction of Aβ or the effects induced intracellularly by Aβ need not be well understood. Various agents that bind to Aβ are capable of inhibiting Aβ neurotoxicity *in vitro,* for example, the Aβ-binding dye, Congo Red, completely inhibits Aβ-induced toxicity in cultured neurons (Yankner et al., 1995). Similarly, the antibiotic rifampacin also prevents Aβ aggregation and subsequent neurotoxicity (Tomiyama et al., 1994). Other compounds are under development as inhibitors of this process either by binding Aβ directly, e.g., hexadecyl-N-methylpiperidinium (HMP) bromide (Wood et al., 1996), or by preventing the interaction of Aβ with other molecules that contribute to the formation of Aβ deposition. An example of such a molecule is heparan sulfate or the heparan sulfate proteoglycan, perlecan, which has been identified in all amyloids and is implicated in the earliest stages of inflammation associated amyloid induction.

Heparan sulfate interacts with the Aβ peptide and imparts characteristic secondary and tertiary amyloid structural features. Recently, small molecule anionic sulfates have been shown to interfere with this reaction to prevent or arrest amyloidogenesis (Kisilevsky, 1995), although it is not evident whether these compounds will enter the CNS. A peptide based on the sequence of the perlecan-binding domain appears to inhibit the interaction between Aβ and perlecan, and the ability of Aβ-derived peptides to inhibit self-polymerization is being explored as a potential lead to developing therapeutic treatments for AD. The effectiveness of these compounds *in vitro,* however, is likely to be modest for a number of reasons, most notably the need for chronic penetration of the blood brain barrier.

As another means of inhibiting or retarding Aβ aggregation, WO 96/25435 discloses the potential for using a monoclonal antibody, which is end-specific for the free C-

terminus of the Aβ 1-42 peptide, but not for the Aβ 1-43 peptide, in preventing the aggregation of Aβ 1-42. While the administration of such an Aβ end-specific monoclonal antibody is further disclosed to interact with the free C-terminal residue of Aβ 1-42, thereby interfering with and disrupting aggregation that may be pathogenic in AD, there is no specific disclosure on how these Aβ C-terminal-specific monoclonal antibodies would be used therapeutically. Although direct or indirect manipulation of Aβ peptide aggregation appears to be an attractive therapeutic strategy, a possible disadvantage of this general approach may be that pharmacological compounds of this class will need to be administered over a long period of time, and may accumulate and become highly toxic in the brain tissue.

WO 98/44955 takes a novel approach to avoiding the problems associated with repeated administration of pharmacological agent and discloses a method for preventing the onset of Alzheimer's Disease or for inhibiting progression of Alzheimer's Disease through the stable ectopic expression in the brain of recombinant antibodies end-specific for amyloid-β peptides.

Recently, Schenk et al. (1999) demonstrated that immunization with amyloid-β attenuated Alzheimer's disease-like pathology in PDAPP transgenic mice serving as an animal model for amyloid-β deposition and Alzheimer's disease-like neuropathologies. They reported that immunization of young animals prior to the onset of Alzheimer's disease-type neuropathologies essentially prevented the development of β-amyloid plaque formation, neuritic dystrophy and astragliosis, whereas treatment in older animals after the onset of Alzheimer's disease-type neuropathologies was observed to reduce the extent and progression of these neuropathologies.

Although the results reported by Schenk et al. provides promise for using immunomodulation as a general approach to treat Alzheimer's disease, immunization with intact amyloid-β according to Schenk et al. has several problems that need to be addressed in developing an immunization program for treatment of Alzheimer's disease in humans. One problem is that

it is not clear how readily one can raise an anti-self antibody response by immunizing humans with human amyloid-β. Moreover, even if an anti-self antibody response is raised against human amyloid-β, it is unclear whether or not auto-immunity might develop which would be injurious to the patient. Other problems include how the immunization would be reversed or halted as the antigen in the form of endogenous amyloid-β is always available to the patients' immune system and whether or not administering amyloid-β, which is believed to be neurotoxic, would have severe and adverse pharmacological effects on the patient.

An alternative to a peptide-based approach is to elucidate the cellular mechanism of Aβ neurotoxicity and develop therapeutics aimed at those cellular targets. The focus has been on controlling calcium dysfunction of free radical mediated neuronal damage. It has been postulated that Aβ binds to RAGE (the receptor for advanced glycation end-products) on the cell surface, thereby triggering reactions that could generate cytotoxic oxidizing stimuli (Yan et al., 1996). Blocking access of Aβ to the cell surface binding site(s) might retard progression of neuronal damage in AD. To date there are no specific pharmacological agents for blocking Aβ-induced neurotoxicity.

Citation of any document herein is not intended as an admission that such document is pertinent prior art, or considered material to the patentability of any claim of the present application. Any statement as to content or a date of any document is based on the information available to applicant at the time of filing and does not constitute an admission as to the correctness of such a statement.

SUMMARY OF THE INVENTION

The present invention provides a chimeric peptide or mixture of chimeric peptides with an end-specific B cell epitope from a naturally-occurring internal peptide cleavage product of a precursor or mature protein, as free N-terminus or C-terminus, fused with or without spacer amino acid residue(s) to a T helper

cell epitope derived from a source different from that of the internal peptide cleavage product.

The present invention also provides an immunizing composition which includes an immunizing effective amount of the chimeric peptide or mixture of chimeric peptides. This immunizing composition is used to raise antibodies in a subject mammal where the internal peptide cleavage product, for which the B cell epitope of the chimeric peptide is end-specific, is a self molecule.

Further provided by the present invention is a method for immunization of a mammal against the free N-terminus or free C-terminus of an internal self peptide cleavage product derived from a self precursor or mature protein. More particularly, the method for immunization is directed to raising antibodies and immunizing individuals against amyloid β peptides associated with amyloid β deposits and plaques.

Yet other aspects of the present invention are directed to a molecule which includes the antigen-binding portion of an antibody specific for the chimeric peptide according to the present invention and a method for passive immunization using this molecule.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a schematic representation of the β-amyloid precursor protein (βAPP) and the products of α, β, γ-secretase cleavage. The general locations of various domains are indicated along with the cleavage sites for α, β, and γ-secretases.

Figure 2 shows a partial amino acid sequence (SEQ ID NO:1) of the region of βAPP from which amyloid-β peptides (Aβ) are derived. The arrows indicate the α-, β-, and γ- secretase cleavage sites.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

One aspect of the present invention is directed generally to a chimeric peptide or a mixture of chimeric peptides in which a N- or C-terminal end-specific B cell epitope

from a naturally-occurring internal peptide cleavage product of a precursor or mature protein, as a free N- or C-terminus, is fused with or without spacer amino acid residue(s) to a T helper cell epitope from a different source. The chimeric peptide or peptides are used in an immunizing composition for immunizing a mammal against the free N-terminus or free C-terminus of an internal peptide cleavage product which is a self molecule of the immunized mammal (molecule native thereto). More specifically as a preferred embodiment of the present invention, the chimeric peptide(s) have an N- or C-terminal end-specific B cell epitope, which is the first two to five amino acid residues of the N-terminus or the last two to five amino acid residues of the C-terminus of an amyloid β peptide, fused to a T helper cell epitope. When such chimeric peptide(s) are administered to a human individual as part of an immunizing composition, that individual will be immunized against the amyloid β peptide or peptides from which the end-specific B cell epitope is derived.

By way of the preferred embodiments of a chimeric peptide containing an amyloid β peptide-specific B cell epitope and a method for immunizing against an amyloid β peptide as examples of the present invention, those of skill in the art will readily appreciate that the chimeric peptides(s), immunizing composition, and method for immunization can be extended and directed to other internal peptide cleavage products, for which end-specific antibodies raised by the present method would not be cross-reactive with the precursor or mature self protein. Similarly, most of the advantages described below for the method for immunization according to the present invention, which raises end-specific antibodies against amyloid β peptides in humans as a preferred embodiment, are generally applicable for immunization with the chimeric peptide or peptides of the present invention.

It is well-known that antibody responses produced by B cells to a defined region of a protein or peptide require that T helper cells of the immune system recognize another part of that antigen simultaneously. This is commonly referred to as B/T cell collaboration. According to the present invention, this

phenomena can be mimicked by making a synthetic chimeric peptide which contains both B and T cell epitopes in a contiguous linear sequence. Such chimeric peptides have been used very successfully to drive antibody production in mice, human/mice chimeras and primates (Sharma et al., 1993; Ifversen et al., 1995; O'Hern et al., 1997).

In the present invention, the B cell epitope containing the first two to five amino acid residues of the free N-terminus or the last two to five amino acid residues of the free C-terminus of an amyloid β peptide is fused, with or without spacer amino acid residues, to a known strong T helper cell epitope to form a chimeric peptide. A non-limiting example of such a known strong T cell epitope is the well-studied tetanus toxoid promiscuous epitope of SEQ ID NO:8 (Ho et al, 1990; Panina-Bordignon et al., 1989) as this epitope is known to work in a number of diverse human genetic backgrounds (Valmori et al., 1992 and 1994).

Immunization with the chimeric peptides(s) containing an amyloid β end-specific B cell epitope and a promiscuous T helper cell epitope of tetanus toxoid, as a preferred embodiment, should give rise to antibodies with the following specificities:

(1) anti-tetanus antibody, which would be irrelevant in humans as most individuals are already sera-positive for tetanus toxoid (i.e., from previous tetanus immunizations);

(2) anti-junction antibodies, which recognize novel epitopes created by the junction joining the end-specific B-cell epitope of an amyloid β peptide and a T helper cell epitope of tetanus toxoid, but would not recognize anything other than the immunogen itself, and therefore would be irrelevant *in vivo* in the immunized individual; and

(3) anti-N-terminal or anti-C-terminal end-specific amyloid β antibodies, which are the desired antibodies sought to be raised by the method according to the present invention for inhibiting, reducing, or even perhaps reversing amyloid β deposit/plaque formation.

The desired anti-N-terminal or anti-C-terminal end-specific amyloid β antibodies raised by the method for immunization according to the present invention are able to discriminate between an amyloid β peptide and the β amyloid protein precursor (βAPP) from which it is proteolytically derived. These end-specific amyloid β antibodies bind specifically to the terminus/end of an amyloid β peptide to slow down, reduce or prevent the accumulation of amyloid β peptides in the extracellular space, interstitial fluid and cerebrospinal fluid of the brain, and the aggregation into senile amyloid deposits or plaques and to block the interaction of amyloid β peptides with other molecules that contribute to the neurotoxicity of amyloid β.

The presence of anti-N-terminal or anti-C-terminal end-specific amyloid β antibodies in the blood and in the extracellular space, interstitial fluid and cerebrospinal fluid of the brain, where soluble amyloid β peptides are present, promotes the formation of soluble antibody-amyloid β complexes. These soluble antibody-amyloid β complexes are cleared from the central nervous system by drainage of the extracellular space, interstitial fluid and cerebrospinal fluid into the general blood circulation through the arachnoid villi of the superior sagittal sinus. In this manner, soluble amyloid β peptides are prevented from accumulating in the extracellular space, interstitial fluid and cerebrospinal fluid to form amyloid deposits and/or to induce neurotoxicity (Fig. 1). Furthermore, clearance of soluble amyloid-β peptides in accordance with the method of the present invention is expected to reduce the inflammatory process observed in Alzheimer's Disease by inhibiting, for example, amyloid-β-induced complement activation and cytokine release, and by also blocking the interaction of Aβ with cell surface receptors such as the RAGE receptor.

As shown in Fig. 1 (see Schehr, 1994), and discussed in the Background Art section, the β-amyloid protein precursor (βAPP) is believed to also serve as a precursor for a proteolytic product, soluble β-amyloid protein precursor (sβAPP), thought to have growth promoting and neuroprotective

functions. It will be readily appreciated by those of skill in the art that the anti-N-terminal or anti-C-terminal end-specific amyloid β antibodies will not interfere with the normal biological functions of βAPP that are not associated with the formation of amyloid β peptides.

The advantages of the method for immunization according to the preferred embodiment of the present invention include:

(1) a cheap peptide antigen, that is readily and easily produced and controlled for quality assurance, is used in active immunization;

(2) inclusion of only two to three, and perhaps up to four or five, amino acid residues from the N- or C- terminus of an internal peptide cleavage product (amyloid β peptide) should minimize the amount of antibody produced which reacts with the precursor or mature protein from which the internal peptide cleavage product is proteolytically derived (βAPP), and hence might interfere with the function of this precursor or mature protein;

(3) use of an independent non-self T cell epitope should break self-tolerance and allow production of antibodies to a self-antigen (Schofield et al., 1991);

(4) the absence in the chimeric peptide(s) of a T cell epitope from the internal peptide cleavage product (amyloid β) should avoid any significant problems of autoimmunity, since anti-self T cell immunity underlies progression of all known autoimmune diseases; and

(5) the immunization should be self-limiting and reversible, with antibody titers gradually falling off with time, since the patient's immune system will never naturally encounter the combination of amyloid β with tetanus toxin as immunogen. This self-limiting and reversible immunization has been demonstrated in clinical trials with synthetic contraceptive vaccines (Talwar et al., 1997).

The chimeric peptide of the present invention is represented by formula (I) or formula (II)

(I) $N-(S)_m-(T_h)_n$

(II) $(T_h)_n-(S)_m-C$

where:

N is the first 2, 3, 4 or 5 amino acid residues from the free N-terminus of a naturally-occurring internal peptide cleavage product, such as an amyloid β peptide, which, when naturally-occurring in a mammal, is derived from a precursor protein or a mature protein;

C is the last 2, 3, 4 or 5 amino acid residues from the free C-terminus of the naturally occurring internal peptide cleavage product;

T_h is a T helper cell epitope derived from a natural source (i.e., species of living organism) different from that of the naturally-occurring internal peptide cleavage product

S is a spacer amino acid residue;

m is 0, 1, 2, 3, 4, or 5; and

n is 1, 2, 3, or 4.

Also contemplated are mixtures of chimeric peptides of formula (I) and formula (II) in which the internal peptide cleavage product of each chimeric peptide can be the same or different, i.e., different amyloid β peptides. These chimeric peptide mixtures are however not limited to mixtures of formula (I) and formula (II) chimeric peptides and can include mixtures of formula (I) peptides, where N is from different internal peptide cleavage products (i.e., different amyloid β peptides), or mixtures of formula (II) peptides, where C is from different internal peptide cleavage products (i.e., different amyloid β peptides).

The chimeric peptide of the present invention has a length between about 20 to 90 amino acid residues, preferably between about 20 to 75 amino acid residues, more preferably between about 20 to 50 amino acid residues, and most preferably between about 20 to 40 amino acid residues.

The B cell epitope from the free N-terminus (N) or the free C-terminus (C) of the naturally-occurring internal peptide cleavage product is preferably two or three amino acid residues, which is not sufficient for raising antibodies cross-reactive with the precursor protein or the mature protein of the peptide

cleavage product. A B cell epitope of four or five amino acid residues from the free N-terminus or the free C-terminus of the naturally-occurring internal peptide cleavage product may also be suitable, although there is a slightly increased possibility that some antibodies may be raised which are cross-reactive with the precursor protein or the mature protein.

A preferred chimeric peptide embodiment of the present invention has a N or C in formula (I) or (II), respectively, from an amyloid β peptide which, when naturally-occurring in humans, is derived from βAPP. As used herein, the term "derived from" means that, when obtained from a natural source, the peptide or protein is the result of cleavage or conversion from a precursor protein or a mature protein, whether by a single proteolytic cleavage event or by more than one cleavage and/or conversion event. In the case of the preferred embodiment, any amyloid β peptide found to occur naturally in amyloid deposits, fibrils, and plaques is encompassed by the term "amyloid β peptide" as the internal peptide cleavage product. Peptides of SEQ ID NOs:2 (Aβ1-40), 3 (Aβ1-42), 4 (Aβ1-43), 5 (Aβ3-42), 6 (Aβ11-42), and 7 (Aβ17-42) are non-limiting examples of such "amyloid β peptides".

T_h is a T helper cell epitope that is derived from a natural source different from the source of the naturally-occurring internal peptide cleavage product. In other words, the T helper cell epitope is not recognized as part of a self-molecule in the mammal subject immunized according to the method of the present invention. The T helper cell epitope is combined with a B cell epitope specific to the N-terminus or C-terminus of peptide cleavage product which is internal (not located at the ends) to a precursor or mature protein to evoke an efficient antibody response.

It is known that immunogens must be presented in conjunction with major histocompatibility (MHC) class II antigens to evoke an efficient antibody response. The MHC class II antigens produced by antigen-presenting cells (APCs) bind to T cell epitopes present in the immunogen in a sequence specific manner. This MHC class II-immunogen complex is recognized by

$CD4^+$ lymphocytes (T_h cells), which cause the proliferation of specific B cells capable of recognizing a B cell epitope from the presented immunogen and the production of B cell epitope-specific antibody by such B cells. Since amyloid β peptides are self molecules, they do not possess any recognizable T_h epitopes, and B cell epitopes of 2 to 5 amino acid residues would lack any T cell epitopes altogether. Such epitopes can be provided by specific sequences derived from potent immunogens including tetanus toxin, pertussis toxin, the measles virus F protein and the hepatitis B virus surface antigen (HBsAg). The T_h epitopes selected are preferably capable of eliciting T helper cell responses in large numbers of individuals expressing diverse MHC haplotypes. These epitopes function in many different individuals of a heterogeneous population and are considered to be promiscuous T_h epitopes. Promiscuous T_h epitopes provide an advantage of eliciting potent antibody responses in most members of genetically diverse population groups.

Moreover, the T helper cell epitopes in the chimeric peptide of the present invention are also advantageously selected not only for a capacity to cause immune responses in most members of a given population, but also for a capacity to cause memory/recall responses. When the mammal is human, the vast majority of human subjects/patients receiving immunotherapy with the chimeric peptide of the present invention will already have been immunized with the pediatric vaccines (i.e., measles+mumps+rubella and diphtheria+pertussis+tetanus vaccines) and, possibly, the hepatitis B virus vaccine. These patients have therefore been previously exposed to at least one of the T_h epitopes present in chimeric pediatric vaccines. Prior exposure to a T_h epitope through immunization with the standard vaccines should establish T_h cell clones which can immediately proliferate upon administration of the chimeric peptide (i.e., a recall response), thereby stimulating rapid B cell responses to the chimeric peptide. In addition, the T_h epitopes avoid any pathogen-specific B cell and/or suppressor T cell epitopes which could lead to carrier-induced immune suppression, a problem

encountered when toxin molecules are used to elicit T helper cell responses.

The T_h epitopes in the chimeric peptide of the invention are promiscuous but not universal. This characteristic means that the T_h epitopes are reactive in a large segment of an outbred population expressing different MHC antigens (reactive in 50 to 90% of the population), but not in all members of that population. To provide a comprehensive, approaching universal, immune reactivity for an internal peptide cleavage product, a combination of chimeric peptides with different T_h epitopes can be prepared. For example, a combination of four chimeric peptides with promiscuous T_h epitopes from tetanus and pertussis toxins, measles virus F protein and HBsAg may be more effective.

Promiscuous T_h epitopes often share common structural features. For example, promiscuous T_h epitopes range in size from about 15 to about 30 residues. Amphipathic helices are a common feature of the T_h epitopes. An amphipathic helix is defined by an α-helical structure with hydrophobic amino acid residues dominating the surrounding faces. T_h epitopes frequently contain additional primary amino acid patterns such as a Gly or a charged reside followed by two to three hydrophobic residues followed in turn by a charged or polar residue. This pattern defines Rothbard sequences. T_h epitopes often obey the 1, 4, 5, 8 rule, where a positively charged residue is followed by hydrophobic residues at the fourth, fifth and eighth positions after the charged residue. Since all of these structures are composed of common hydrophobic, charged and polar amino acids, each structure can exist simultaneously within a single T_h epitope.

T_h is therefore a sequence of amino acids (natural or non-natural) that contain a T_h epitope. A T_h epitope can be a continuous or discontinous epitope. Hence, not every amino acid of T_h is necessarily part of the epitope. Accordingly, T_h epitopes, including analogs and segments of T_h epitopes, are capable of enhancing or stimulating an immune response to the internal peptide cleavage product. Immunodominant T_h epitopes

are broadly reactive in animal and human populations with widely divergent MHC types (Celis et al., 1988; Demotz et al., 1989; and Chong et al., 1992). The T_h domain of the chimeric peptides of the present invention has from about 10 to about 50 amino acids residues and preferably from about 10 to about 30 amino acids residues. When multiple T_h epitopes are present (i.e., $n \geq 2$), then each T_h epitope is independently the same or different.

T_h epitope analogs include substitutions, deletions and insertions of one to about five amino acid residues in the T_h epitope. T_h segments are contiguous portions of a T_h epitope that are sufficient to enhance or stimulate an immune response to the internal peptide cleavage product. An example of T_h segments is a series of overlapping peptides that are derived from a single longer peptide.

The T_h epitopes of the present invention include hepatitis B surface antigen T helper cell epitopes (HB_s T_h), pertussis toxin T helper cell epitopes (PT T_h), tetanus toxin T helper cell epitopes (TT T_h), measles virus F protein T helper cell epitope (MV_{F1} T_h), *Chlamydia trachomitis* major outer membrane protein T helper cell epitopes (CT T_h), diphtheria toxin T helper cell epitopes (DT T_h), *Plasmodium falciparum* circumsporozoite T helper cell epitopes (PF T_h), *Schistosoma mansoni* triose phosphate isomerase T helper cell epitopes (SM T_h), *Escherichia coli* TraT T helper cell epitopes (TraT T_h) and immune-enhancing analogs and epitope sequences are provided below in Table 1.

TABLE 1

TT_o T_h	SEQ ID NO:8
HB_3 T_h	SEQ ID NO:9
TT_1 T_h	SEQ ID NO:10
TT_1 T_h	SEQ ID NO:11
TT_2 T_h	SEQ ID NO:12
PT_{1A} T_h	SEQ ID NO:13
TT_3 T_h	SEQ ID NO:14
PT_2 T_h	SEQ ID NO:15
MV_{F1} T_h	SEQ ID NO:16
MV_{F2} T_h	SEQ ID NO:17
TT_4 T_h	SEQ ID NO:18
TT_5 T_h	SEQ ID NO:19
CT_1 T_h	SEQ ID NO:20
DT_1 T_h	SEQ ID NO:21
DT_2 T_h	SEQ ID NO:22
PF T_h	SEQ ID NO:23
SM T_h	SEQ ID NO:24
$TraT_1$ T_h	SEQ ID NO:25
$TraT_2$ T_h	SEQ ID NO:26
$TraT_3$ T_h	SEQ ID NO:27

Immunogenicity can be improved through the addition of spacer residue(s) S (i.e. Gly-Gly) between the promiscuous T_h epitope and the B cell epitope of the chimeric peptide according to the present invention. In addition to physically separating the T_h epitope from the B cell epitope, the glycine spacer residues can disrupt any artificial secondary structures created by the joining of the T_h epitope with the B cell epitope, and thereby eliminate interference between the T and/or B cell responses. The conformational separation between the helper epitope and the antibody eliciting domain thus permits more efficient interactions between the presented immunogen and the appropriate T_h and B cells. The amino acid residue(s) for S can

be naturally-occurring amino acids or non-naturally-occurring amino acids, which include, but are not limited to, β-alanine, ornithine, norleucine, norvaline, hydroxyproline, thyroxine, gamma-amino butyric acid, homoserine, citrulline and the like.

The chimeric peptides of the present invention can be made by synthetic chemical methods which are well known to the ordinarily skilled artisan. Hence, the chimeric peptides can be synthesized using the automated Merrifield techniques of solid phase synthesis with either t-Boc or F-moc chemistry on Peptide Synthesizers such as an Applied Biosystems Peptide Synthesizer.

After complete assembly of the desired chimeric peptide, the resin is treated according to standard procedures to cleave the peptide from the resin and deblock the protecting groups on the amino acid side chains. The free peptide is purified by HPLC and characterized biochemically, for example, by amino acid analysis or by sequencing. Purification and characterization methods for peptides are well-known to one of ordinary skill in the art.

Alternatively, the longer linear chimeric peptides can be synthesized by well-known recombinant DNA techniques. Any standard manual on DNA technology provides detailed protocols to produce the chimeric peptides of the invention. To construct a gene encoding a chimeric peptide of the present invention, the amino acid sequence is reverse transcribed into a nucleic acid sequence, and preferably using optimized codon usage for the organism in which the gene will be expressed. Next, a synthetic gene is made, typically by synthesizing overlapping oligonucleotides which encode the peptide and any regulatory elements, if necessary. The synthetic gene is inserted in a suitable cloning vector and recombinant clones are obtained and characterized. The chimeric peptide is then expressed under suitable conditions appropriate for the selected expression system and host, and the chimeric peptide is purified and characterized by standard methods.

As an optional segment to the chimeric peptide, an immunostimulatory epitope of the invasin protein of a *Yersinia* species can be linked to the T helper cell epitope of the

chimeric peptide opposite from the B cell epitope. The invasins of the pathogenic bacteria Yersinia spp. are outer membrane proteins which mediate entry of the bacteria into mammalian cells (Isberg et al., 1990). Invasins of cultured mammalian cells by the bacterium was demonstrated to require interaction between the Yersinia invasin molecule and several species of the β1 family of integrins present on the cultured cells (Tran Van Nhieu et al., 1991) Since T lymphocytes are rich in β1 integrins (especially activated immune or memory T cells) the effects of invasin on human T cell have been investigated (Brett et al., 1993). It is thought that integrins facilitate the migration of immune T cells out of the blood vessels and through connective tissues to sites of antigenic challenge through their interaction with extracellular matrix proteins including fibronectin, laminin and collagen. The carboxy-terminus of the invasin molecule was found to be co-stimulatory for naive human $CD4^+$ T in the presence of the non-specific mitogen, anti-CD3 antibody, causing marked proliferation and expression of cytokines. The specific invasin domain which interacts with the β1 integrins to cause this stimulation also was identified (Brett et al., 1993). Because of the demonstrated T cell co-stimulatory properties associated with this domain, it can be linked to the promiscuous T_h epitope in the chimeric peptide of the present invention opposite from the B cell epitope.

Many of the outer membrane proteins of Gram-negative bacteria are both lipid-modified and very immunogenic. Because of the apparent correlation between covalent lipid linkage and immunogenicity, tripalmitoyl-S-glycerol cysteine (Pam_3Cys), a lipid common to bacterial membrane proteins, can be coupled to synthetic peptides representing either B cell or cytotoxic T cell epitopes. Because significant adjuvanting responses are elicited by this lipid linkage, promiscuous T_h epitope of the chimeric peptide can be lipid modified opposite its linkage to the B cell epitope. Such lipid-modified chimeric peptides are likely to be more immunogenic than the unmodified version of the same peptide.

U.S. Patent 5,843,446, which includes a disclosure of T_h epitopes and the immunostimulatory properties of invasin epitopes and lipid moieties, is herein incorporated entirely by reference.

Immunogenicity can further be significantly improved if the antigens are co-administered with adjuvants. Adjuvants enhance the immunogenicity of an antigen but are not necessarily immunogenic themselves. Adjuvants may act by retaining the antigen locally near the site of administration to produce a depot effect facilitating a slow, sustained release of antigen to cells of the immune system. Adjuvants can also attract cells of the immune system to an antigen depot and stimulate such cells to elicit immune responses.

Immunostimulatory agents or adjuvants have been used for many years to improve the host immune responses, e.g. to vaccines. Intrinsic adjuvants, such as lipopolysaccharides, normally are the components of the killed or attenuated bacteria used as vaccines. Extrinsic adjuvants are immunomodulators which are typically non-covalently linked to antigens and are formulated to enhance the host immune responses. Thus, adjuvants have been identified that enhance the immune response to antigens delivered parenterally. Some of these adjuvants are toxic, however, and can cause undesirable side-effects, making them unsuitable for use in humans and many animals. Indeed, only aluminum hydroxide and aluminum phosphate (collectively commonly referred to as alum) are routinely used as adjuvants in human and veterinary vaccines. The efficacy of alum in increasing antibody responses to diptheria and tetanus toxoids is well established and a HBsAg vaccine has been adjuvanted with alum as well.

A wide range of extrinsic adjuvants can provoke potent immune responses to antigens. These include saponins complexed to membrane protein antigens (immune stimulating complexes), pluronic polymers with mineral oil, killed mycobacteria in mineral oil, Freund's complete adjuvant, bacterial products, such as muramyl dipeptide (MDP) and lipopolysaccharide (LPS), as well as lipid A, and liposomes. To efficiently induce humoral

immune responses (HIR) and cell-mediated immunity (CMI), immunogens are emulsified in adjuvants. Many adjuvants are toxic, inducing granulomas, acute and chronic inflammations (Freund's complete adjuvant, FCA), cytolysis (saponins and Pluronic polymers) and pyrogenicity, arthritis and anterior uveitis (LPS and MDP). Although FCA is an excellent adjuvant and widely used in research, it is not licensed for use in human or veterinary vaccines because of its toxicity.

U.S. Pat. No. 4,855,283 teaches glycolipid analogues including N-glycosylamides, N-glycosylureas and N-glycosylcarbamates, each of which is substituted in the sugar residue by an amino acid, as immuno-modulators or adjuvants. U.S. Pat. No. 4,258,029 teaches that octadecyl tyrosine hydrochloride (OTH) functions as an adjuvant when complexed with tetanus toxoid and formalin inactived type I, II and III poliomyelitis virus vaccine. Also, Nixon-George et al., 1990, reported that octadecyl esters of aromatic amino acids complexed with a recombinant hepatitis B surface antigen enhanced the host immune responses against hepatitis B virus.

The addition of exogenous adjuvant/emulsion formulations which maximize immune responses to the internal peptide cleavage product are preferred. The adjuvants and carriers that are suitable are those: (1) which have been successfully used in Phase I human trials; (2) based upon their lack of reactogenicity in preclinical safety studies, have potential for approval for use in humans; or (3) have been approved for use in food and companion animals.

Immunotherapy regimens which produce maximal immune responses following the administration of the fewest number of doses, ideally only one dose, are highly desirable. This result can be approached through entrapment of immunogen in microparticles. For example, the absorbable suture material poly(lactide-co-glycolide) co-polymer can be fashioned into microparticles containing immunogen. Following oral or parenteral administration, microparticle hydrolysis *in vivo* produces the non-toxic byproducts, lactic and glycolic acids, and releases immunogen largely unaltered by the entrapment

process. The rate of microparticle degradation and the release of entrapped immunogen can be controlled by several parameters, which include (1) the ratio of polymers used in particle formation (particles with higher co-glycolide concentrations degrade more rapidly); (2) particle size, (smaller particles degrade more rapidly than larger ones); and, (3) entrapment efficiency, (particles with higher concentrations of entrapped antigen degrade more rapidly than particle with lower loads). Microparticle formulations can also provide primary and subsequent booster immunizations in a single administration by mixing immunogen entrapped microparticles with different release rates. Single dose formulations capable of releasing antigen ranging from less than one week to greater than six months can be readily achieved. Moreover, delivery of the chimeric peptide according to the present invention entrapped in microparticles can also provide improved efficacy when the microparticulate immunogen is mixed with an exogenous adjuvant/emulsion formulations.

The efficacy of the chimeric peptides can be established and analyzed by injecting an animal, e.g., mice or rats, with the chimeric peptide formulated in alum and then following the immune response to the internal peptide cleavage product.

Another aspect of the present invention provides an immunizing composition which includes an immunizing effective amount of one or more of the chimeric peptides of the invention and a pharmaceutically acceptable carrier, excipient, diluent, or auxiliary agent, including adjuvants. Accordingly, the chimeric peptides can be formulated as an immunizing composition using adjuvants, pharmaceutically-acceptable carriers, excipients, diluents, auxiliary agents or other ingredients routinely provided in immunizing compositions. Such formulations are readily determined by one of ordinary skill in the art and include formulations for immediate release and for sustained release, e.g., microencapsulation. The present immunizing compositions can be administered by any convenient route including subcutaneous, oral, intramuscular, or other

parenteral or internal route. Similarly the vaccines can be administered as a single dose or divided into multiple doses for administration. Immunization schedules are readily determined by the ordinary skilled artisan. For example, the adjuvants or emulsifiers that can be used in this invention include alum, incomplete Freund's adjuvant, liposyn, saponin, squalene, L121, emulsigen and ISA720. In preferred embodiments, the adjuvants/emulsifiers are alum, incomplete Freund's adjuvant, a combination of liposyn and saponin, a combination of squalene and L121 or a combination of emulsigned and saponin.

The immunizing compositions of the present invention contain an immunoeffective amount of one or more of the chimeric peptides and a pharmaceutically acceptable carrier. Such compositions in dosage unit form can contain about 0.5 μg to about 1 mg of each peptide per kg body weight. When delivered in multiple doses, the dosage unit form is conveniently divided into the appropriate amounts per dosage.

Immunizing compositions which contain cocktails of two or more of the chimeric peptides of the present invention enhance immunoefficacy in a broader population and thus provide a better immune response against the internal peptide cleavage product, such as amyloid β. Other immunostimulatory synthetic chimeric peptide immunogens are arrived at through modification into lipopeptides so as to provide built-in adjuvanticity for potent vaccines. The immune response to synthetic chimeric peptide immunogens of the present invention can be improved by delivery through entrapment in or on biodegradable microparticles of the type described by O'Hagan et al (1991). The immunogens can be encapsulated with or without adjuvant, including covalently attached lipid moiety such as Pam_3Cys, and such microparticles can be administered with an immunostimulatory adjuvant such as Freund's Incomplete Adjuvant or alum. The microparticles function to potentiate immune responses to an immunogen and to provide time-controlled release for sustained or periodic responses. for oral administration, and for topical administration (O'Hagan et al., 1991).

A further aspect of the present invention is a method for immunization against the free N-terminus and/or free C-terminus of a naturally-occurring internal peptide cleavage product, which, when naturally-occurring in a mammal, is derived from a precursor protein or a mature protein. This method according to the present invention involves administering to a mammal an immunizing composition containing the chimeric peptides of the present invention for which the internal peptide cleavage product is a self molecule. A preferred embodiment of this method is one in which the method is directed to immunizing against amyloid β peptides to raise anti-N-terminal or anti-C-terminal end-specific amyloid β antibodies which do not cross-react with the β amyloid precursor protein. In this preferred embodiment, the N and/or C of formulas (I) and (II) of the chimeric peptide(s) used in the present method are from the N-terminus and/or C-terminus of amyloid β peptides.

A still further aspect of the present invention is a molecule which contains the antigen-binding portion of an antibody, preferably a monoclonal antibody, specific for the chimeric peptide according to the present invention. This molecule can be an antibody raised against the chimeric peptide *in vivo* or can be a recombinant antibody that is intended to encompass fragments of antibodies containing the antigen-binding portion, chimeric or humanized immunoglobulin molecules of any isotype, as well as a single-chain antibodies.

Chimeric antibodies are understood to be molecules, different portions of which are derived from different animal species, such as those humanized antibodies having a variable region derived from a mouse monoclonal antibody and a human immunoglobulin constant region. Chimeric antibodies and methods for their production are well known in the art. For example, the DNA encoding the variable region of the antibody can be inserted into or joined with DNA encoding other antibodies to produce chimeric antibodies (U.S. patent 4,816,567; Orlandi et al., 1989).

Single-chain antibodies can be single chain composite polypeptides having end-specific peptide binding capability and

comprising a pair of amino acid sequences homologous or analogous to the variable regions of an immunoglobulin light and heavy chain (linked V_H-V_L or single chain Fv). Both V_H and V_L may copy natural monoclonal antibody sequences, or one or both of the chains may comprise a CDR-FR construct of the type described in U.S. Patent 5,091,513. The separate polypeptides analogous to the variable regions of the light and heavy chains are held together by a peptide linker. Methods of production of such single chain antibodies, e.g., single chain Fv (scFv), particularly where the DNA encoding the polypeptide structures of the V_H and V_L chains are characterized or can be readily ascertained by sequence analysis, may be accomplished in accordance with the methods described, for example, in U.S. Patent 4,946,778, U.S. Patent 5,091,513, U.S. Patent 5,096,815, Biocca et al. (1993), Duan et al. (1994), Mhashilkar et al. (1995), Marasco et al. (1993), and Richardson et al. (1995).

Besides the conventional method of raising antibodies *in vivo*, antibodies can be produced *in vitro* using Phage Display technology. The production of recombinant antibodies is much faster compared to conventional antibody production and they can be generated against an enormous number of antigens. In contrast, in the conventional method, many antigens prove to be non-immunogenic or extremely toxic, and therefore cannot be used to generate antibodies in animals. Moreover, affinity maturation (i.e., increasing the affinity and specificity) of recombinant antibodies is very simple and relatively fast. Finally, large numbers of different antibodies against a specific antigen can be generated in one selection procedure. To generate recombinant monoclonal antibodies one can use various methods all based on Phage Display libraries to generate a large pool of antibodies with different antigen recognition sites. Such a library can be made in several ways: One can generate a synthetic repertoire by cloning synthetic CDR3 regions in a pool of heavy chain germline genes and thus generating a large antibody repertoire, from which recombinant antibody fragments with various specificities can be selected. One can use the lymphocyte pool of humans as starting material

for the construction of an antibody library. It is possible to construct naive repertoires of human IgM antibodies and thus create a human library of large diversity. This method has been widely used successfully to select a large number of antibodies against different antigens.

Yet another aspect of the present invention is a method for passive immunization involving administering to a mammal, preferably human, the molecule which contains the antigen-binding portion of an antibody specific for the chimeric peptide according to the present invention for which the internal peptide cleavage product is a self molecule. A preferred embodiment of this method is one in which the method is directed to passively immunizing with molecules in which the antigen-binding portion thereof is end-specific for amyloid β peptides.

Having now fully described this invention, it will be appreciated by those skilled in the art that the same can be performed within a wide range of equivalent parameters, concentrations, and conditions without departing from the spirit and scope of the invention and without undue experimentation.

While this invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications. This application is intended to cover any variations, uses, or adaptations of the inventions following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth as follows in the scope of the appended claims.

All references cited herein, including journal articles or abstracts, published or corresponding U.S. or foreign patent applications, issued U.S. or foreign patents, or any other references, are entirely incorporated by reference herein, including all data, tables, figures, and text presented in the cited references. Additionally, the entire contents of

the references cited within the references cited herein are also entirely incorporated by references.

Reference to known method steps, conventional methods steps, known methods or conventional methods is not in any way an admission that any aspect, description or embodiment of the present invention is disclosed, taught or suggested in the relevant art.

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art (including the contents of the references cited herein), readily modify and/or adapt for various applications such specific embodiments, without undue experimentation, without departing from the general concept of the present invention. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance presented herein, in combination with the knowledge of one of ordinary skill in the art.

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WHAT IS CLAIMED IS:

1. A chimeric peptide represented by formula (I) or formula (II),

(I) $N-(S)_m-(T_h)_n$

(II) $(T_h)_n-(S)_m-C$

or chimeric peptides which are mixtures of formula (I) peptides, mixtures of formula (II) peptides, or mixtures of formula (I) and formula (II) peptides, wherein:

N is the first 2, 3, 4, or 5 amino acid residues from the free N-terminus of a naturally-occurring internal peptide cleavage product which, when naturally-occurring in a mammal, is derived from a precursor protein or a mature protein;

C is the last 2, 3, 4, or 5 amino acid residues from the free C-terminus of said naturally-occurring internal peptide cleavage product;

T_h is a T helper cell epitope;

S is a spacer amino acid residue;

m is 0, 1, 2, 3, 4, or 5; and

n is 1, 2, 3, or 4.

2. The chimeric peptide or peptides according to claim 1, wherein said internal peptide cleavage product is an amyloid β peptide, which, when naturally-occurring, is derived from cleavage of β amyloid precursor protein (βAPP).

3. The chimeric peptide or peptides according to claim 2, wherein said internal peptide cleavage product has an amino acid sequence selected from the group consisting of SEQ ID NOs:2, 3, 4, 5, 6, 7, and mixtures thereof.

4. The chimeric peptide or peptides according to claim 1, wherein N is the first 2 or 3 amino acid residues from the free N-terminus of said internal peptide cleavage product.

5. The chimeric peptide or peptides according to claim 1, wherein C is the last 2 or 3 amino acid residues from the free C-terminus of said internal peptide cleavage product.

6. The chimeric peptide or peptides according to claim 1, wherein T_h is a promiscuous T helper cell epitope.

7. The chimeric peptide or peptides according to claim 6, wherein said promiscuous T helper cell epitope is derived from tetanus toxin, pertussis toxin, diphtheria toxin, measles virus F protein, hepatitis B virus surface antigen, *Chlamydia trachomitis* major outer membrane protein, *Plasmodium falciparum* circumsporozoite, *Schistosoma mansoni* triose phosphate isomerase, or *Escherichia coli* TraT.

8. The chimeric peptide or peptides according to claim 7, wherein said promiscuous T helper cell epitope has an amino acid sequence selected from the group consisting of SEQ ID NOs:8 to 27.

9. The chimeric peptide or peptides according to claim 1, wherein S is glycine.

10. An immunizing composition, comprising an immunizing effective amount of the chimeric peptide or peptides according to claim 1 and a pharmaceutically acceptable carrier, excipient, diluent, or auxiliary agent.

11. The immunizing composition according to claim 10, wherein said pharmaceutically acceptable auxiliary agent is an adjuvant.

12. The immunizing composition according to claim 11, wherein said adjuvant is alum.

13. A method for immunization against the free N-terminus or free C-terminus of an internal self peptide cleavage product derived from a precursor protein or a mature protein, comprising administering to a mammal the immunizing composition according to claim 10, for which the internal peptide cleavage product is a self molecule of the mammal.

14. The method according to claim 13, wherein the mammal is a human.

15. The method according to claim 14, wherein the internal self peptide cleavage product is an amyloid β peptide, which when naturally-occurring, is derived from cleavage of β amyloid precursor protein, whereby said method raises antibodies specific to the free N-terminus and/or free C-terminus of the amyloid β peptide.

16. A molecule comprising the antigen-binding portion of an antibody specific for the chimeric peptide according to claim 1.

17. The molecule according to claim 16, wherein said antibody is a monoclonal antibody.

18. A method for passive immunization, comprising administering to a mammal the molecule of claim 16.

19. The method according to claim 18, wherein the mammal is human.

20. The method according to claim 19, wherein said chimeric peptide against which the antibody is raised is one where the internal peptide cleavage product is an amyloid β peptide, which, when naturally-occurring, is derived from cleavage of β amyloid precursor protein (βAPP).

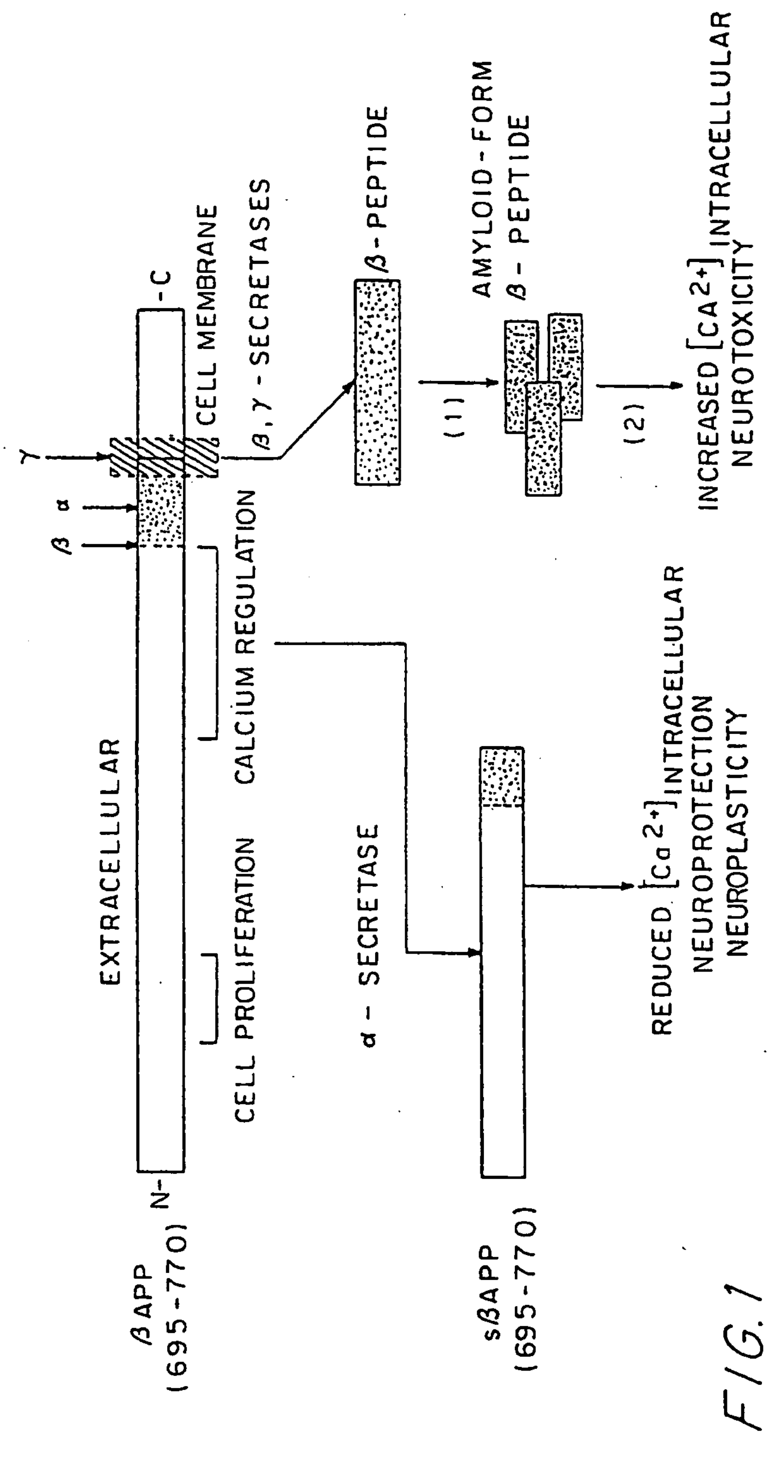
βAPP (695-770)
N-
-C
EXTRACELLULAR
β
α
γ
CELL MEMBRANE
CELL PROLIFERATION
CALCIUM REGULATION
β, γ - SECRETASES
β - PEPTIDE
(1)
AMYLOID - FORM β - PEPTIDE
(2)
INCREASED [CA 2+] INTRACELLULAR NEUROTOXICITY
α - SECRETASE
sβAPP (695-770)
REDUCED [Ca 2+] INTRACELLULAR
NEUROPROTECTION
NEUROPLASTICITY

FIG. 1

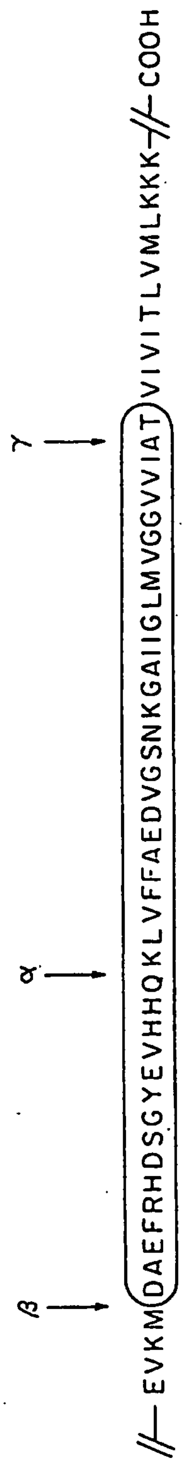
β
α
γ
EVKM
DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIAT
VIVITLVMLKKK
COOH

FIG. 2

SEQUENCE LISTING

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<130> CHAIN8A

<140> NOT YET ASSIGNED
<141> 2000-12-08

<150> 60/169,687
<151> 1999-12-08

<160> 27

<170> PatentIn version 3.0

<210> 1
<211> 59
<212> PRT
<213> Human

<400> 1

Glu Val Lys Met Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val
1 5 10 15

His His Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys
20 25 30

Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Val
35 40 45

Ile Val Ile Thr Leu Val Met Leu Lys Lys Lys
50 55

<210> 2
<211> 40
<212> PRT
<213> Human

<400> 2

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys
1 5 10 15

Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile
20 25 30

Gly Leu Met Val Gly Gly Val Val
35 40

<210> 3
<211> 42
<212> PRT
<213> Human

<220>
<221> misc_feature
<223> Xaa is L-Asp, D-Asp, or L-iso Asp

<400> 3

Xaa Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys
1 5 10 15

Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile
20 25 30

Gly Leu Met Val Gly Gly Val Val Ile Ala
35 40

<210> 4
<211> 43
<212> PRT
<213> Human

<400> 4

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys
1 5 10 15

Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile
20 25 30

Gly Leu Met Val Gly Gly Val Val Ile Ala Thr
35 40

<210> 5
<211> 40
<212> PRT
<213> Human

<220>
<221> misc_feature
<223> Xaa is pyroglutamate

<400> 5

Xaa Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys Leu Val
1 5 10 15

Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile Gly Leu
20 25 30

Met Val Gly Gly Val Val Ile Ala
35 40

<210> 6
<211> 32
<212> PRT
<213> Human

<220>
<221> misc_feature
<223> Xaa is pyroglutamate

<400> 6

Xaa Val His His Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser
1 5 10 15

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Asn Lys Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala
            20                  25                  30

<210>  7
<211>  26
<212>  PRT
<213>  Human

<400>  7

Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile
1               5                   10                  15

Gly Leu Met Val Gly Gly Val Val Ile Ala
            20                  25

<210>  8
<211>  14
<212>  PRT
<213>  Tetanus toxin bacteria

<400>  8

Gln Tyr Ile Lys Ala Asn Ser Lys Phe Ile Gly Ile Thr Glu
1               5                   10

<210>  9
<211>  15
<212>  PRT
<213>  Hepatitis B virus

<400>  9

Phe Phe Leu Leu Thr Arg Ile Leu Thr Ile Phe Gln Ser Leu Asp
1               5                   10                  15

<210>  10
<211>  30
<212>  PRT
<213>  Pertussis toxin bacteria

<400>  10

Lys Lys Leu Arg Arg Leu Leu Tyr Met Ile Tyr Met Ser Gly Leu Ala
1               5                   10                  15

Val Arg Val His Val Ser Lys Glu Glu Gln Tyr Tyr Asp Tyr
            20                  25                  30

<210>  11
<211>  17
<212>  PRT
<213>  Tetanus toxin bacteria

<400>  11

Lys Lys Gln Tyr Ile Lys Ala Asn Ser Lys Phe Ile Gly Ile Thr Glu
1               5                   10                  15

Leu
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<210> 12
<211> 22
<212> PRT
<213> Tetanus toxin bacteria

<400> 12

Lys Lys Phe Asn Asn Phe Thr Val Ser Phe Trp Leu Arg Val Pro Lys
1 5 10 15

Val Ser Ala Ser His Leu
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<210> 13
<211> 15
<212> PRT
<213> Pertussis toxin bacteria

<400> 13

Tyr Met Ser Gly Leu Ala Val Arg Val His Val Ser Lys Glu Glu
1 5 10 15

<210> 14
<211> 27
<212> PRT
<213> Tetanus toxin bacteria

<400> 14

Tyr Asp Pro Asn Tyr Leu Arg Thr Asp Ser Asp Lys Asp Arg Phe Leu
1 5 10 15

Gln Thr Met Val Lys Leu Phe Asn Arg Ile Lys
20 25

<210> 15
<211> 24
<212> PRT
<213> Pertussis toxin bacteria

<400> 15

Gly Ala Tyr Ala Arg Cys Pro Asn Gly Thr Arg Ala Leu Thr Val Ala
1 5 10 15

Glu Leu Arg Gly Asn Ala Glu Leu
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<210> 16
<211> 15
<212> PRT
<213> Measles virus

<400> 16

Leu Ser Glu Ile Lys Gly Val Ile Val His Arg Leu Glu Gly Val
1 5 10 15

<210> 17
<211> 20
<212> PRT
<213> Measles virus

<400> 17

Gly Ile Leu Glu Ser Arg Gly Ile Lys Ala Arg Ile Thr His Val Asp
1 5 10 15

Thr Glu Ser Tyr
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<210> 18
<211> 17
<212> PRT
<213> Tetanus toxin bacteria

<400> 18

Trp Val Arg Asp Ile Ile Asp Asp Phe Thr Asn Glu Ser Ser Gln Lys
1 5 10 15

Thr

<210> 19
<211> 16
<212> PRT
<213> Tetanus toxin bacteria

<400> 19

Asp Val Ser Thr Ile Val Pro Tyr Ile Gly Pro Ala Leu Asn His Val
1 5 10 15

<210> 20
<211> 25
<212> PRT
<213> Chlamydia trachomitis

<400> 20

Ala Leu Asn Ile Trp Asp Arg Phe Asp Val Phe Cys Thr Leu Gly Ala
1 5 10 15

Thr Thr Gly Tyr Leu Lys Glu Asn Ser
20 25

<210> 21
<211> 23
<212> PRT
<213> Diphteria toxin bacteria

<400> 21

Asp Ser Glu Thr Ala Asp Asn Leu Glu Lys Thr Val Ala Ala Leu Ser
1 5 10 15

Ile Leu Pro Gly Ile Gly Cys
20

<210> 22
<211> 39
<212> PRT
<213> Diphteria toxin bacteria

<400> 22

Glu Glu Ile Val Ala Gln Ser Ile Ala Leu Ser Ser Leu Met Val Ala
1 5 10 15

Gln Ala Ile Pro Leu Val Gly Glu Leu Val Asp Ile Gly Phe Ala Ala
20 25 30

Thr Asn Phe Val Glu Ser Cys
35

<210> 23
<211> 21
<212> PRT
<213> Plasmodium falciparum

<400> 23

Asp Ile Glu Lys Lys Ile Ala Lys Met Glu Lys Ala Ser Ser Val Phe
1 5 10 15

Asn Val Val Asn Ser
20

<210> 24
<211> 17
<212> PRT
<213> Schistoma mansoni

<400> 24

Lys Trp Phe Lys Thr Asn Ala Pro Asn Gly Val Asp Glu Lys Ile Arg
1 5 10 15

Ile

<210> 25
<211> 14
<212> PRT
<213> Escherichia coli

<400> 25

Gly Leu Gln Gly Lys Ile Ala Asp Ala Val Lys Ala Lys Gly
1 5 10

<210> 26
<211> 19
<212> PRT
<213> Escherichia coli

<400> 26

Gly Leu Ala Ala Gly Leu Val Gly Met Ala Ala Asp Ala Met Val Glu
1 5 10 15

Asp Val Asn

<210> 27
<211> 20
<212> PRT

<213> Escherichia coli

<400> 27

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Ser Thr Glu Thr Gly Asn Gln His His Tyr Gln Thr Arg Val Val Ser
1               5                   10                  15

Asn Ala Asn Lys
            20
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